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(57) Abstract

Novel peptides which incorporate novel amino acid, halo-olefin dipeptide isosteres, and/or cyclic tripeptides are useful in the diagnosis, prophylaxis, treatment and management of autoimmune diseases.

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NOVEL IMMUNOSUPPRESSANTS

This invention relates to certain novel peptides which incorporate novel amino acids, halo-olefin dipeptide isosteres, and/or cyclic tripeptides, and which are useful in the diagnosis, prophylaxis, treatment and management of autoimmune diseases.

Normally, the body's own ("self") cells do not trigger an immune response. It is believed that one way in which the immune response is mediated is by selective presentation of antigens to T-cells by identifying molecules, the production of which is mediated by a gene sequence known as the major histocompatibility complex ("MHC"). The nature of these MHC molecules may vary depending on the type and location of the cell as well as on the type of antigen presented. When an MHC molecule presents an antigen to a T-cell, the T-cell apparently recognizes the antigen-MHC molecule complex, and an immune response ensues. If self antigens are presented, there is ordinarily no population of T cells recognizing such antigens, and no immune response occurs. In some cases, however, presentation of self antigens can lead to an inappropriate, autoimmune, response.

A number of diseases are believed to have an autoimmune component and, indeed, have been associated with particular MHC molecules. One object of this invention is to provide blocker compounds which inactivate the MHC molecules associated with various autoimmune diseases. The MHC blockers of this invention have primarily been evaluated in connection with rheumatoid arthritis, which has been associated with the MHC alleles HLA-DR1, HLA-DR4w4, and/or HLA-DR4w14. Other autoimmune diseases which may be treated,

prevented or relieved using the peptides of this invention include ankylosing spondylitis, Hashimoto's disease, multiple sclerosis, Sjogren syndrome, scleroderma, polymyositis, dermatomyositis, systemic lupus erythematosus, juvenile rheumatoid arthritis, juvenile diabetes, myasthenia gravis, bullous pemphigoid, pemphigus, glomerulonephritis, Goodpasture's syndrome, autoimmune hemolytic anemia, pernicious anemia, idiopathic thrombocytopenic purpura, Grave's disease, Addison's disease, graft rejections and the like, as well as autoimmune diseases of animals.

In the past, treatment of autoimmune diseases has focused on relieving the outward symptoms of the disease, using drugs designed to relieve the pain, inflammation, and so forth associated with the immune response. Such drugs, however, which in the case of rheumatiod arthritis include corticosteroids, anti-malarial drugs, gold salts, and nonsteroidal anti-inflammatory drugs, have proved, in many cases, to be ineffective in the treatment of autoimmune diseases or to have serious side effects or both. Conventional immuno-suppressants which work systemically to suppress the entire immune system have the disadvantage of increasing the risk of cancers and opportunistic infections, as well as other side effects. There is thus a clear and long felt need for immunosuppressants which are relatively nontoxic and which can be used to treat the specific autoimmune response associated with particular autoimmune diseases. The MHC blocker peptides of this invention meet that need.

Peptides which bind to MHC were known in the art prior to this invention. For example, the binding characteristics of immunogenic peptides such as influenza hemaglutinin (HA), ragweed protein and tetanus toxin have been extensively studied. Such immunogenic peptides, however, are not useful as immunosuppressants. Additionally, such peptides generally only bind well to a single type of MHC receptor, such as DR1.

The challenge is therefore to develop peptides which bind to the desired MHC receptors competitively with the antigens to be blocked, but which do not themselves stimulate T cell proliferation. Moreover, for the blocker peptides to be useful as pharmaceuticals, they should be relatively stable in vivo. This is generally a problem with peptides, which tend to have poor bioavailability due to lack of stability against proteases under physiologic conditions and rapid excretion by the kidneys. Moreover, due to the fact that new MHC molecules are constantly forming on the surface of antigen presenting cells, it is particularly important for an MHC blocker to be available over a substantial period of time so as to be able to bind to the newly formed MHC molecules. It has now surprisingly been discovered that incorporation of particular novel unnatural residues at specified points in the MHC blocker peptide will enhance the bioavailability and stability of the peptide and will inhibit T-cell activation by masking or blocking selected MHC molecules. The novel peptides are shown in vivo to be useful in the propylaxis and treatment of disease, particularly autoimmune disease.

The unnatural residues of the invention may be novel amino acids, halo-olefin dipeptide isosteres, or cyclic tripeptides, all as more fully described herein. Incorporation of these residues at selected points in the molecule appears to alter the configuration or presentation of the non-MHC binding portion of peptide without significantly changing the configuration or presentation of the side chains which are critical to MHC binding, thereby permitting or even enhancing MHC binding, and at the same time interfering with or eliminating recognition of the peptide by T-cells or by peptidases.

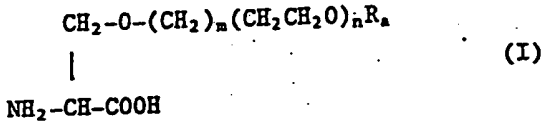
Finally, peptides of the invention which have a strong affinity for MHC molecules associated with particular autoimmune diseases are also useful in a variety of diagnostic assays to determine the presence of MHC molecules associated with autoimmune disease in the early stages of the disease, before the outward symptoms of the

disease become apparent. Such early diagnosis aids greatly in the treatment and management of autoimmune disease. Peptides of the invention can also be used in competitive screening assays to measure MHC binding to other molecules of interest.

The present invention thus provides stable, soluble, relatively nontoxic compounds which are antagonistic to MHC molecules associated with autoimmune diseases and which are not immunogenic. In particular, the invention provides novel peptides incorporating novel, unnatural residues, which peptides show a strong affinity for HLA-DR molecules, especially DR1, DR4w4 and DR4w14, the molecules associated with rheumatoid arthritis. These novel peptides have proved stable under physiologic conditions and are relatively nontoxic in vivo. The development of these novel compounds represents a significant advance in the potential for treatment of those afflicted with or at risk for autoimmune diseases. Moreover, the invention provides novel residues which are useful in other applications of peptide chemistry in that they confer unexpectedly useful stability and resorption properties to peptides in which they are incorporated.

The novel amino acids of the invention are as follows:

AA-I is an O-substituted serine of formula I



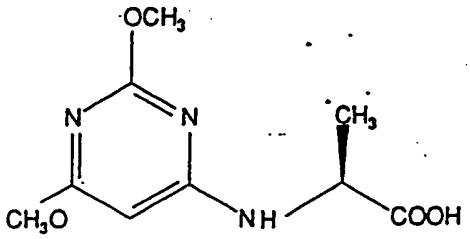
(I)

in which m is 0 or 1, n is 1-7 and R_a is C_{1-5} alkyl, provided that when m=0 and n=3, R_a is not methyl:

Although the nonnatural amino acid O-triethyleneglycol serine monomethylether (the compound of formula I in which m is zero, n is

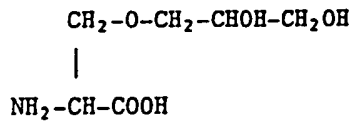
three, and R_a is methyl) is not novel per se, it may be utilized in place of AA-I in the MHC blocker peptides of the invention, and MHC blocker peptides incorporating this amino acid are novel.

AA-II is N-[6-(2,4-dimethoxypyrimidyl)] alanine of formula II:



(II)

AA-III is O-(2,3-dihydroxypropyl) serine of formula III:



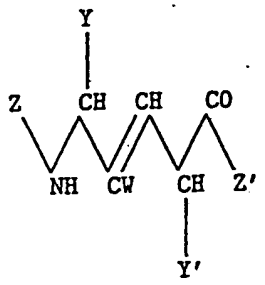
(III)

Although the foregoing novel amino acids are depicted in free acid form, it is understood that may also be in the form of their acid or base addition salts, or be in protected form.

The novel halo-olefin peptide isosteres are peptides in which the peptide bond has been replaced with a halo-olefin group, preferably a fluoro-ethylene group (-{CF=CH}-) or a chloro-ethylene group (-{CCl=CH}-). It is unexpected and extremely significant that the novel halo-olefin peptide isosteres of the invention can be substituted for peptide bonds even at or near the binding regions of the peptides without destroying the binding activity of the peptides. Although the halo-olefin group thus mimics an amide bond's geometry and electron profile, the halo-olefin group is not susceptible to

attack by proteases. The halo-olefin dipeptide isosteres of this invention may, in principle, be incorporated into any peptide without altering the geometry of the peptide or the chirality of the amino acid residues. The advantages of and processes for making these peptide isosteres are thus not limited to MHC blocking peptides, but could be utilized to enhance the stability, binding activity and bioavailability of a wide variety of biologically active peptides in addition to the ones exemplified herein.

Specifically, the novel halo-olefin peptide isosteres comprise residues of two or more amino acids (other than isosteres of diglycine and phenylalanyl glycine) linked by a halo-olefin group as shown in Formula IV:



IV

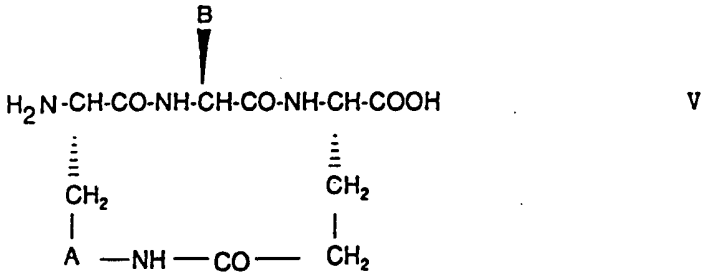
where W is a halogen, preferably fluorine or chlorine;
Z represents an amino acid residue, a peptide, a protective group, or hydrogen;
Z′ represents an amino acid residue in protected or unprotected form, a peptide, in protected or unprotected form, a protective group, or hydroxyl;
Y and Y′ represent side chains of α-amino acid residues, (e.g., where the halo-peptide isostere is an isostere of phenylalanyl alanine, for example, Y is C_6H_5-CH_2- and Y′ is CH_3-); and
the configuration of the olefin bond is preferably trans (E).

Compounds of formula IV may be in free form or in the form of their acid or base addition salts, or be in protected form at the

N-terminus, the C-terminus or at both.

Halo-olefin dipeptide isosteres in which both amino acid residues have chiral centers and halo-olefin isosteres of peptides having more than two amino acid residues are entirely novel. Although fluoro-olefin isosteres of glycine-glycine and phenylalanine-glycine are known, such isosteres have not previously been used in formulating MHC blocker peptides, therefore MHC blocker peptides incorporating these isosteres are novel. The process for making these halo-olefin isosteres, disclosed herein, is novel and has the significant advantage that chirality of the amino acid residues is controlled, which is not possible using previously known methods.

The cylic tripeptides of the invention, in free acid form, are of formula V:



V

wherein A is a linear C_{2-4} alkylene group, which may be interrupted by an oxygen atom, and B is the side chain of an αamino acid, preferably of phenylalanine (i.e., benzylmethyl) or alanine (i.e., methyl). When A is the group $-(CH_2)_3-$ and B is methyl, for example, the peptide of formula V is the tripeptide Lys-Ala-Glu in which a further peptide bond has been formed between the ε-amino group of the lysine residue and the γ-carboxy group of the glutamic acid residue. The compound of formula V may be in free form or in the form of its acid or base addition salts,

or be in protected form at the N-terminus, the C-terminus or at both.

The novel MHC antagonist compounds described herein are small peptides having eight to seventeen residues of amino acids, and comprising one or more of the novel residues of Formulas I, II, III, IV, or V, arranged from the N-terminus to the C-terminus according to Formula VI:

$$NX\text{-}R_1\text{-}P\text{-}R_2\text{-}T\text{-}R_3\text{-}CX \quad (VI)$$

wherein NX is an N-terminal protective group, a residue of a D-amino acid, a residue of an amino acid in protected form, or is not present;
R_1 is a series of from zero to three amino acid residues;
P is a hydrophobic L-amino acid residue or, where NX and R_1 are not present, may be a hydrophobic carboxylic acid residue;
R_2 is a series of three to five L-amino acid residues;
T is a an amino acid residue selected from the group containing L-threonine, L-serine, L-proline, and L-hydroxyproline;
R3 is a series of two to five amino acid residues; and
CX is a residue of an amino acid, an amino acid amide, or an amino alcohol.

The preferred MHC blocker peptides of the invention are those where NX, R_1, P, R_2, T, R_3, and CX of Formula VI are, independently, as follows:

NX represents an N-terminal protective group, a residue of a D-amino acid, a residue of an amino acid in protected form, or is not present. An N-terminal protective group is defined as a group which tends to protect peptides from degradation in vivo, including such groups as alkyls, cycloalkyls, aryls, arylalkyls, acyls, polyethylene glycols and polyethylene glycol carboxylic acids, pyroglutamic acid, succinyl, methoxy succinyl, benzoyl phenylacetyl, 2-, 3- or 4-

pyridylalkanoyl, aroyl, alkanoyl (including acetyl and cycloalkanoyl), arylalkanoyl (including pyridyls), arylaminocarbonyl, alkylaminocarbonyl, cycloaminocarbonyl, alkyloxycarbonyl (e.g., carbamate caps, especially butyloxycarbonyl (Boc)), cycloalkoxycarbonyl, and fluorenylmethoxycarbonyl (Fmoc) among others.

NX is preferably butyloxycarbonyl, fluorenylmethoxycarbonyl, or a residue of D-proline, D-tyrosine, D-alanine, polyethylene glycol carboxylic acid, O-triethyleneglycol serine monomethylether, AA-I, or AA-II.

R_1 is a series of from zero to three amino acid residues, preferably L-amino acid residues. Where R_1 is present, it preferably contains residues of one or more of the following amino acids: alanine, lysine, O-triethyleneglycol serine monomethylether, AA-I, AA-II, or AA-III.

P is a hydrophobic L-amino acid residue selected from the group containing L-phenylalanine and hydrogenated analogues thereof (especially cyclohexylalanine), napthylalanine and hydrogenated analogs thereof, and trimethylsilylalanine (TMSA), or is a residue of a hydrophobic carboxylic acid, preferably cyclohexylpropanoic acid or adamantylacetic acid. Where P is a residue of a carboxylic acid, NX and R_1 are preferably not present.

R_2 is a series of any three to five L-amino acid residues. R2 preferably contains four amino acids selected from the group containing glycine, L-threonine, L-alanine, L-lysine, amino-isobutyric acid, O-triethyleneglycol serine monomethylether, AA-I, AA-III, or contains the cyclic tripeptide residue of Formula V and one other L-amino acid. When R2 contains the cyclic tripeptide of Formula V, the tripeptide is preferably adjacent to T, and the residue in R2 adjacent to P is preferably an L-amino acid, most

preferably L-valine. The cyclic tripeptide may also span R_1-P-R_2, e.g., as -Lys-Phe-Glu-, or -Orn-Phe-Glu-, and where this is the case, R_2 may contain an additional two to three residues, e.g., -Ala-Lys- or -Ala-Ala-Lys-.

T is a residue of L-threonine, L-serine, L-proline, or L-hydroxyproline, preferably L-threonine or L-trans-4-hydroxyproline, most preferably L-threonine.

R3 is a series of two to five amino acid residues, preferably L-amino acid residues, and most preferably residues of the L-forms of one or more of the following amino acids: alanine, leucine, lysine, phenylalanine, O-triethyleneglycol serine monomethylether, AA-I, or AA-III. Where R2 comprises the cyclic tripeptide of Formula V, R3 is preferably Leu-Lys-Ala.

CX is a residue of an amino acid, an amino acid amide, or an amino alcohol, preferably L-alaninol, L-threoninol, D-phenylalanineamide or D-alanineamide.

One or more amide bond may be replaced with halo-olefin groups at any position in the peptide. Particularly preferred isosteres are -Ala-{CF=CH}-Ala-, -Phe-{CF=CH}-Ala-, -Cha-{CF=CH}-Ala- and -Ala-{CF=CH}-Lys-, where Cha designates a cyclohexylalanine residue. Also preferred are the chloro-ethylene analogues of these preferred fluoro-ethylene dipeptide isosteres. When the isostere is -Phe-{CF=CH}-Ala- or -Cha-{CF=CH}-Ala, the Phe or Cha portion of the isostere is P and the Ala portion is the first residue of R_2. When the isostere is -Ala-{CF=CH}-Lys-, it is preferably located in R_2 adjacent to T.

The term "peptide isostere" as used herein refers to a moiety that i) conformationally and functionally serves as a substitute for two or more amino acid residues in a peptide of the present

invention, permitting the interaction of the peptide with its MHC receptor, and ii) differs from a peptide in that one or more amide bonds is replaced with a bond or entity that conformationally and functionally serves as a substitute for an amide bond, such as the halo-olefin group described herein. Peptide isosteres and peptides incorporating peptide isosteres may be synthesized and screened for effect on binding in a variety of assays, e.g., in a competition assay with an unsubstituted parent peptide. For a general discussion of known peptide isosteres, see, Morgan and Gainor, Ann. Repts. Med. Chem. 24:243-252 (1989).

The nomenclature used to describe the peptides of the invention follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxy group is presented to the right (the C-terminus) of each amino acid residue. Amino acid residues may be represented by their standard three letter abbreviations, in which the L-form has the first letter capitalized and the D-form does not. Residues having no chiral center (e.g., glycine, α-amino isobutyric acid, and AA-IV) are classed as L-amino acids for the purposes of defining the invention claimed herein.

The term "amino acid residues" is considered to include the amino acid residue analogues in peptide isosteres, as well as conventional amino acid residues linked by amide bonds. For example, the fluoro-ethylene isostere of dialanine is considered to consist of two alanine residues linked by a fluoro-ethylene group and may be expressed as "-Ala-(CF=CH)-Ala-". Likewise, the term "peptide" as used herein is broadly defined to include (i) such peptide isosteres, and (ii) peptides which are modified at the N- or C-terminals as described herein, as well as peptides comprising amino acid residues linked by amide bonds.

The peptides of the invention may be synthesized from natural or unnatural amino acids, using conventional methods of peptide

synthesis or on an inert polymeric support peptide synthesizer, and purified using reversed-phase high-performance liquid chromatography (HPLC). The purity of the peptides may be confirmed by amino acid sequence and/or composition analysis using conventional means.

Once the desired chain sequence of amino acid residues is obtained, the N-terminal of the peptide may be capped by conventional means with the capping groups disclosed above. The C-terminal of the peptide may be reduced to the amino alcohol or may be amido-substituted in accord with the above described structures, again using methods well known to one skilled in the art.

SCREENING ASSAY METHODS

The affinity of the novel peptides for the HLA-DR molecules and the stability of the peptides under physiological conditions may be assessed in a variety of ways, which are known to one skilled in the art, generally by labelling the peptide with a radionuclide, enzyme, fluorescer, or other labeller, and determining the specific binding activity to the MHC molecule in question. Stability may be measured in vitro as a half-life in minutes under physiologic conditions (e.g., in human serum at 37° C).

Binding to MHC: The procedure used to determine relative specific activities such as those given in the Examples below is a time-resolved fluorescence assay which measures the relative binding activity of the novel peptides to human MHC HLA-DR1, -DR4w4, and -DR4w14, using essentially the following methods:

EBV-transformed homozygous cell lines are used as a source of DR molecules. Cell lines are routinely monitored for DR expression by FACS analysis. Their DR types are confirmed by serological typing and RFLP analysis, using conventional methods. Cell lines used are maintained in vitro by culture in RMPI 1640 medium, supplemented with 10% heat inactivated fetal calf serum or horse serum. Large

quantities of cells are grown in 1 liter roller bottles. Cells are then lysed at a concentration of 10^8 cells/ml in PBS pH 7.2, containing 1% NP40, 5 mM sodium thoranadate, 25 mM iodoacetamide, and 1 mM PMSF. The lysates are cleared of nuclei and debris by centrifugation at 100,000 x g for 45 minutes. DR molecules are purified using the monoclonal antibody LB3.1, covalently coupled to CNBr-Sepharose CL-4B. Epstein-Barr virus transformed human B cell lines, homozygous at the DR locus, are used as a source of DR molecules. Aliquots of cell lysates equivalent to approximately 5 grams of cells are passed sequentially through the following columns: Sepharose CL-4B (10 ml), protein A-Sepharose (5 ml), LB3.1-protein A-Sepharose (15 ml), using a flow rate of 30 ml/h. The columns are washed with 10 column volumes of PBS, 1% n-octylglucoside, 0.05% NP40 (5 ml/hr); 2 column volumes of PBS and 1% octylglucoside. The DR is eluted with 0.05 M diethylamine in 0.15 M NaCl containing 1% octylglucoside and 0.05% NP40 (pH 10.5), immediately neutralized with 2 M glycine pH 2.0 and concentrated by ultra-filtration through an Amicon pM-30 membrane. Protein content is evaluated by a BCA protein assay and confirmed by SDS PAGE electrophoresis and Western blotting.

The purified DR molecules (10-1,000 nM) are incubated with 10 nM biotin labelled or ^{125}I radiolabelled peptide for 16-48 hours in the presence of added protease inhibitors. The final concentrations of protease inhibitors may be, for example, as follows: 1 mM PMSF, 1.3 mM ortho-phenanthroline, 73 µM pepstatin A, 8 mM EDTA, 6 mM N-ethyl maleimide, and 200 µM Nα-p-tosyl -L-lysine chloromethyl ketone (TLCK). The final detergent concentration in the incubation mixture is 0.05% NP-40. One or more of the peptides screened may be hydrophobic and require DMSO to maintain solubility and reduce peptide adsorption to surfaces. In these instances peptide stock solutions are prepared in neat DMSO, and the final DMSO concentration in the incubation mixture is adjusted to 5%. Control experiments may be performed to demonstrate that the IC 50% values obtained with or without DMSO are similar. The DR-peptide complexes are separated

from free peptide by use of a monoclonal anti-DR antibody coupled to microtiter plates. The amount of peptide bound to the MHC is determined in the case of a biotin labelled assay by use of europium-labelled streptodine and time-resolved fluorescence, or by measurement of radiation in the case of a radio-labelled assay. Competitive inhibition by unlabeled peptide is used to determine IC 50% values. The data are expressed as relative binding values.

Cellular Assay for Inhibition of Antigen Presentation: An EBV positive, DR1+ homozygous typing cell line, LK-2, is washed 3X with Hank's Balanced Salt Solution (HBSS), suspended to 5 x 10^6/ml in HBSS, and fixed with 0.5% p-formaldehyde (w/v) at room temperature for 20 minutes. The fixed LG-2 cells are washed 1X with HBSS, washed 2X with RPMI media supplemented with L-glutamine, non-essential amino acids, sodium pyruvate, antibiotics, and 10% human sera type AB (Complete Media, CM), resuspended at 10^6/ml in CM, and then plated as 100 µl into individual wells of a 96 well microtiter dish. The fixed LG-2 cells are then pulsed simultaneously for 2 hours at 37°C with 50 µl of test peptide and 50 µl of stimulatory peptide which have been dissolved in CM. The amount of stimulatory peptide added to each test well is constant and is calculated so as to yield 60-80% of the maximal T cell proliferative response. The potential inhibitor peptide is evaluated in duplicate at several concentrations corresponding to multiples of the stimulatory peptide concentration, usually 3X, 10X, 50X, and 250X. At the end of the two hour incubation the plates are centrifuged, and the media carefully aspirated and replaced with fresh medium. The plates are washed in this manner three times in order to ensure complete removal of unbound peptide. At the end of the washes 50 µl of CM remains in the well. In separate wells, increasing concentrations of stimulatory peptide are assayed in the absence of inhibitory peptide in order to obtain dose-response curves for the stimulatory peptide and to ensure that the amount of stimulatory peptide used in test wells is truly limiting.

Antigen-specific T cells are washed 2X in CM, suspended to 2 x 10^5/ml in CM, and plated as 150 µl into the wells containing the peptide-pulsed LG-2 cells. The cultures are incubated for three days at 37°C, and pulsed with 0.1 µCi/well of ^{3}H-thymidine during the last 16 hours of culture. At the termination of the cultures the cells are harvested onto glass fiber and the amount of ^{3}H-thymidine, which has been incorporated by the responder T cell, determined using liquid scintillation counting.

The inhibitory activity of each peptide is determined relative to a reference peptide of known activity. For each test peptide the amount of inhibition is plotted as a function of the inhibitor concentration, and this graph is used to determine a 50% inhibitory dose. The inhibitory activity is then expressed as the ratio of the 50% inhibitory dose of the standard peptide (determined in a parallel experiment) to that of the test peptide. These data are presented in the last three columns of Table 1. This inhibitory value constitutes an additional means by which the MHC binding peptides can be compared.

Immunogenicity in vivo: C57B1/6 mice are immunized subcutaneously at the tail base and into the hind footpads with 100, 10, 1, or 0.1 nM/mouse of antigen emulsified in CFA. Eight days after immunization, lymph nodes draining the injection site are removed and 4 x 10^5 cells are cultured in wells of microtiter plates in HL-1 medium supplemented with 2 mM L-glutamine, 50 µM 2-ME and 50 µM/ml gentamicin with the indicated antigen concentrations. Cultures are set up in triplicate from each mouse and from pooled lymph node cells. cultures are incubated for three days in a humidified atmosphere of 5% CO_2 in air and were pulsed 10 hours before harvesting with 1µCi [^{3}H]TdR 840Ci/mM). Incorporation of [^{3}H]TdR is measurted by liquid scintillation spectrometry. An immunogenic compound will prime the cultures at levels less than or equal to 1 nM per mouse in CPA. Compounds of the invention which were tested show

immunogenicity only at levels of 100 nM per mouse or higher, and can therefore be considered nonimmunogenic in vivo.

Stability: To analyze the stability of the peptides, the half life of the peptides is determined using a 25% human serum assay. The reaction medium is prepared by mixing 1 part human serum with 3 parts cell culture medium (RPMI), vortexing the mixture, filtration through a 0.45µ filter, and incubation at 37°C for 20 min. The test peptide (0.5mg) is dissolved in 50µl·DMSO, and 10µl of this solution is diluted with 1.5ml of the reaction medium and left to stand at 37°C. Samples of 100µl of the mixture are taken at intervals and added to 200µl of ice-cold 6% aqueous trichloroacetic acid left to stand in ice for 15 min and finally centrifuged for 2 min. at 13000 rpm. 100µl Samples of the supernatant are analysed by HPLC, and a graph is plotted of the % remaining peptide as a function of time. The result is expressed either as the time reqired to reach 50% of the initial peptide concentration, or, for the more stable peptides, as the % of peptide remaining after 24 hr.

GALENIC FORMS

It will be apparent to one skilled in the art that a small, stable peptide of the structure described herein lends itself well to a variety of galenic applications. The drug can be delivered parenterally, orally, nasally, topically, by aerosol, or transdermally. Suitable carriers would be obvious to one skilled in the art.

In the preferred method of administration, the drug is administered parenterally, in a sterile solution, preferably an aqueous solution such as water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid, or the like. The composition may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents,

tonicity adjusting agents, wetting agents and the like. The concentration of peptide in the solution may vary widely and will depend on the desired fluid volume, viscosity, etc., in accordance with the particular mode of administration selected. The peptides of the invention may be combined with one another or with other chemotheraputic agents for increased efficacy. The final solution should be sterile, sterilization being accomplished by well known conventional means, especially sterile filtration. The solution may be packaged for use as is or may be lypholized for subsequent mixture with a sterile solution prior to administration. By way of example; a typical pharmaceutical composition for intravenous infusion could contain on the order of 5% peptide by volume in sterile Ringer's solution.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, or other pharmaceutically acceptable solid carrier. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is one or more peptides of the invention, alone or in combination with other pharmaceutical agents, preferably 25%-75% of one or more of the peptides of the invention. It should be noted that oral administration is not generally suitable for peptides having only amide bonds, but may be preferred for certain peptide isoteres, e.g., the halo-olefin peptide isosteres of this invention.

For aerosol administration, the peptides of the invention are preferably supplied in finely divided form along with a surfactant and a propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Appropriate

aerosol carriers will be well known to one skilled in the art.

The compositions containing the peptides of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from an autoimmune disease, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications, this amount being defined as a therapeutically effective dose. The therapeutically effective dose will depend on the severity and nature of the disease and on the weight and general state of the patient, but generally range from about 0.1 mg to about 2000 mg of peptide per day for a 70 kg patient, with dosages of from about 0.5 mg to about 1000 mg of peptide per day being more commonly used.

In prophylactic applications, compositions containing the peptides of the invention are administered to a patient susceptible to or otherwise at risk of a particular autoimmune disease to enhance the patient's own immunoregulatory capabilities, in an amount defined top be a prophylactically effective dose. The prophylactically effective dose again depends on the nature of the disease and the patient's weight and general health, but generally ranges from about 0.1 mg to about 500 mg per 70 kilogram patient, more commonly from about 0.5 mg to about 200 mg per 70 kg of body weight.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the peptide of the invention sufficient to effectively treat the patient.

DIAGNOSTIC ASSAYS

The peptides may also find use as diagnostic reagents. For example, a peptide may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In such instances, cells bearing the selected MHC antigen, e.g., leukocytes, will be obtained from an individual, incubated in vivo with the peptide(s), an immunostimulatory peptide, and a collection of T cells from the same individual. The proliferation of T cells can then be assayed in the presence or absence of the immunomodulatory peptide or peptide mixture.

EXAMPLES

Example 1: Peptide synthesis

Peptides of the invention may be synthesized by any conventional method of peptide synthesis. Solid state synthesis is preferred, and suitable methods may be found in any standard textbook on peptide synthesis, e.g., as described by Atherton & Shephard, "Solid Phase Peptide Synthesis, a Practical Approach", IRL Press, 1989.

The following example is a solid state synthesis using Fmoc protecting groups, but alternative methods of peptide synthesis would be apparent to one skilled in the art:

222mg 4-(2',4'-Dimethoxyphenyl-Fmoc-amino-methyl)-phenoxy resin (Novabiochem) is deprotected with 50% piperidine/DMF, washed three times with DMF, methanol, DMF and then coupled to a solution of a 2.5 fold excess of the appropriate Fmoc-protected amino acid or isostere in DMF for two hours at room temperature using a suitable mixture of reagents such as 2-(1H-Benzotriazol-1-yl) 1,1,3,3-tetramethyluronium tetrafluoroborate and 1-hydroxybenzotriazol in the presence of N-ethyldiisopropylamine. The resin is washed three times with DMF and methanol. A ninhydrin test establishes that the coupling is complete. The resin is then quelled with DMF, deprotected with 50% piperidine in DMF and the next Fmoc-amino acid or isostere in DMF added. This procedure is repeated until the desired sequence had been assembled.

The peptide is cleaved from the resin by treatment with 2.0ml trifluoroacetic acid and 0.1ml water for 90 minutes at room temperature, filtered, the residue washed with trifluoroacetic acid and the trifluoroacetic acid removed as far as possible by passing a stream of argon over the the surface. The peptide is precipitated by adding 30ml diethyl ether and centrifuged. A further 30ml diethyl ether is added and this procedure repeated four times. The peptide

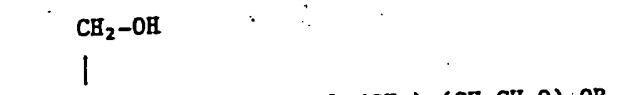
is stirred with 30ml ethyl acetate / diethyl ether (1.5:1), centrifuged and this proceedure repeated once more. It is then dried in a stream of argon, dissolved in water using an ultrasonic bath, filtered and lyophillised. The peptide is purified using HPLC (250 x 21.4mm Dynamax column RP-18, 12µM Spherical 300A, gradient 90% acetronitrile / 10% water / 0.1% trifluoroacetic acid increasing to 25% acetontrile / 75% water / 0.1% trifluoroacetic acid), the fractions collected (UV detector 200nm) and lyophillized. The peptide is then converted to its acetate by dissolving in 10% aqueous acetic acid and passing through a Bio Rad A G4-X4 column. The fractions are collected, lyophillised, and analysed by standard procedures.

The peptide may be protected and deprotected by standard means. The N-terminus may be protected by standard caps or may be a carboxylic acid rather than an amino acid, as described elsewhere herein. When C-terminus modifications are desired, they may be performed after the peptide is synthesized, or, alternatively, the initial starting resin may be varied. The above starting resin yields alanylamide at the C-terminus. To obtain a peptide having a C-terminus threoninol, for example, the above synthesis is performed starting with 187mg. threoninol-p-benzyloxybenzylalcohol resin (Kilo-labor) instead of the 4-(2',4'-dimethoxyphenyl-Fmoc-amino-methyl)-phenoxy resin.

Examples 2-4: Synthesis of novel amino acids

Example 2: Synthesis of AA-I:

AA-I may be synthesized by reacting a compound of formula VII with a compound of formula VIII



VII VIII

in which X is a protecting group, Hal is a halogen, preferably bromine, and m, n, and R_a are as stated above, in the presence of a strong base, for example sodium hydride, to obtain an O-substituted serine of the invention, substantially as follows:

a) [O-2-(2-methoxyethoxy)ethyl]serine (AA I where m=0, n=2, and R_a is methyl)

To a solution of 10g BOC-serine in 200ml DMF is added 4.5g sodium hydride and 7ml 1-bromo-2-(2-methoxyethoxy)ethane, and the mixture is allowed to stand at room temperature for 12 hr. The mixture is concentrated under vacuum to about ¼ of the original volume, and is partitioned between ethyl acetate and saturated aqueous potassium bisulphate solution. The organic phase is separated, washed twice with water, dried and evaporated to give 11g of the BOC-protected product.

The BOC-group is removed by treating the product with 4N hydrochloric acid at room temperature for 2 hr. Evaporation and vacuum drying gives the title product as the hydrochloride.

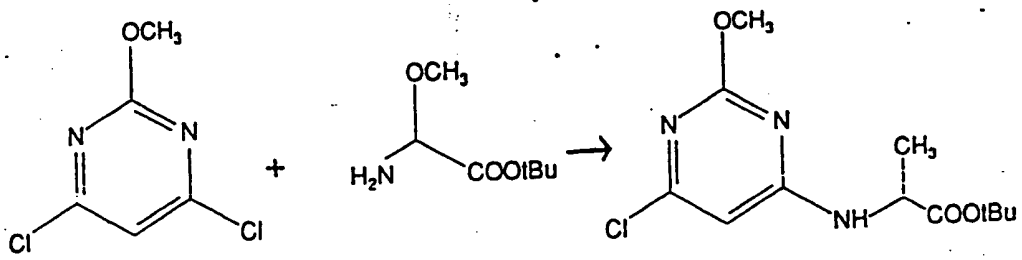
b) FMOC-[O-2-(2-methoxyethoxy)ethyl]serine

The hydrochloride obtained above is dissolved in a mixture of 160ml water and 100ml dioxane containing 16g sodium hydroxide, the solution cooled to 0-5°C, and slowly treated with 11g FMOC-chloride. The reaction mixture is allowed to warm to room temperature over 3hr, acidified with conc. hydrochloric acid, and extracted with ethyl

acetate. The crude product is purified by chromatography using as mobile phase 6:4 ethyl acetate/hexane acidified by shaking with 5% conc. hydrochloric acid. The yield of pure product is 5.6g.

Example 3: Synthesis of AA-II:

AA-II may be produced by reacting 2-methoxy-4,6-dichloro-pyrimidine of formula IX with alanine t-butyl ester of formula X



IX X XI

in an inert solvent e.g. dioxane and in the presence of a weak base, e.g. triethylamine. The product XI is then reacted with sodium methoxide in methanol to remove the remaining chlorine and ester groups, yielding the compound of formula II. To join AA-II to the peptide chain, the carboxy group of AA-II may be linked to an amino acid benzyl ester, preferably phenylalanine benzyl ester, then the benzyl group may be removed to permit linkage to the remaining peptide chain. The process is substantially as follows:

a) N-[6-(2-methoxy-4-chloropyrimidyl)]alanine t-butyl ester

2-Methoxy-4,6-dichloropyrimidine (21.8 g, 122 mmol), alanine t-butyl ester (19.5 g, 135 mmol) and triethylamine (25 ml, 185 mmol) are added to 150 ml dioxane and stirred for 3 days at room temperature. The reaction mixture is filtered from the precipitated $Et_3N.HCl$, evaporated, taken up in ethyl acetate and washed with 5% aqueous sodium bicarbonate. The washings are reextracted with ethyl

acetate, and the combined extracts washed with brine, dried over $MgSO_4$, filtered and evaporated. The resulting oily residue (30 g) is chromatographed on 1.2kg Kieselgel (0.063 - 0.200 mm) with hexane/ether to give 75 mmol of the title product and 42 mmol of the starting material, which can be re-used.

b) N-[6-(2,4-dimethoxypyrimidyl)]alanine (AA-II)

To a solution of sodium (5g, 217 matom) in 200 ml absolute methanol is added the product of a) above (21g, 73 mmol), dissolved in a little abs. methanol. The mixture is placed in a pressure vessel and kept at 130°C overnight. The reaction mixture is evaporated, taken up in water and extracted with ether to remove unchanged starting material. The aqueous phase is adjusted to pH 3-4 with acetic acid, extracted three times with ethyl acetate, washed with brine, dried over $MgSO_4$, filtered and evaporated to give the title product as an oil (15g). M.p. of DCHA salt 154.5 - 156.5°C.

c) (N-[6-(2,4-dimethoxypyrimidyl)]alanyl)phenylalanine benzyl ester

The product of step b), in the form of the DCHA salt, (5g, 12.25 mmol) is dissolved in 250 ml dichloromethane. To this solution is added at 0°C L-phenylalanine benzyl ester in free base form (3.3g, 13 mmol) dissolved in 50 ml dichloromethane, together with BOP-Cl (3.4g, 13.5 mmol), and the mixture is stirred at room temperature overnight. The precipitate of BOP DCHA salt is filtered off, and the filtrate is diluted with ethyl acetate and washed first with potassium bicarbonate then with 10% phosphoric acid, then with brine. The solution is finally dried over $MgSO_4$, filtered and evaporated.

Purification by chromatography on Kieselgel using 3:1 hexane/ethyl acetate as eluent gives 4.5g of an oil which can be shown by NMR to consist of a mixture of two diastereoisomers in the ratio of 1:1, thus proving that the product of step b) is a racemate.

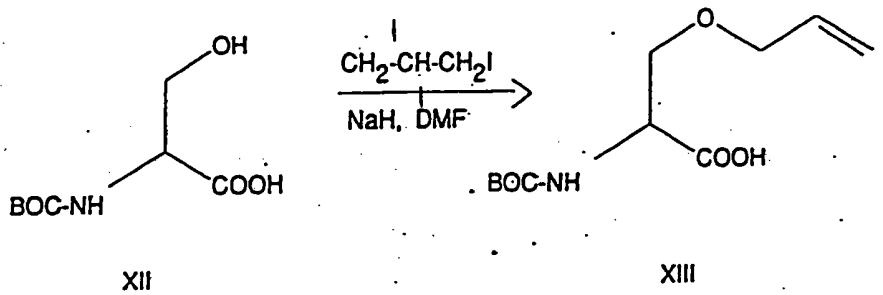
Chromatographic separation of the two diastereoisomers is not possible at this stage, but becomes possible when the benzyl group is removed.

d) (N-[6-(2,4-dimethoxypyrimidyl)]alanyl)phenylalanine

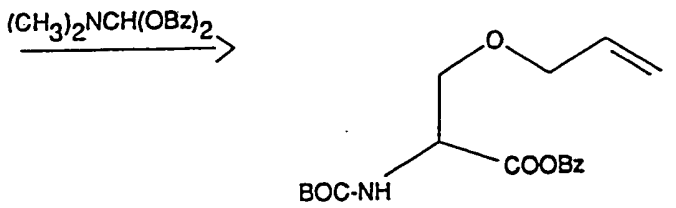
The product of step c) (4.5g, 9.7 mmol) is hydrogenated in 200 ml ethanol at room temperature in the presence of 0.5g of a 10% Pd/C catalyst. After one hour no further hydrogen is taken up. The reaction mixture is filtered through Hyflo/talc and evaporated to give 3g of an oily residue. Chromatographic separation on 440g Kieselgel (0.040 - 0.063 mm) using as eluent a mixture of DCM/MeOH/AcOH 380:20:1 gave 1.1g of diastereoisomer A and 0.9g of diastereoisomer B of the title dipeptide.

Example 4: Synthesis of AA-III

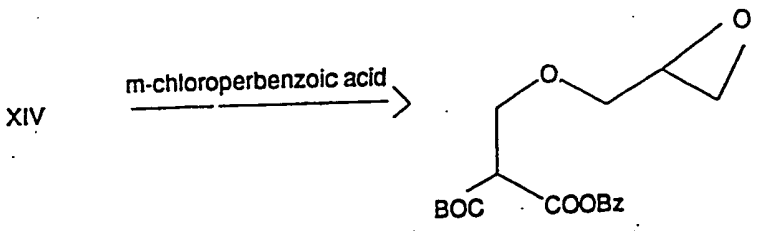
AA-III may be prepared by the following reaction scheme:



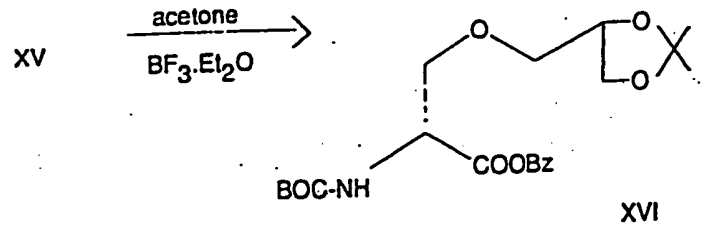
XII XIII



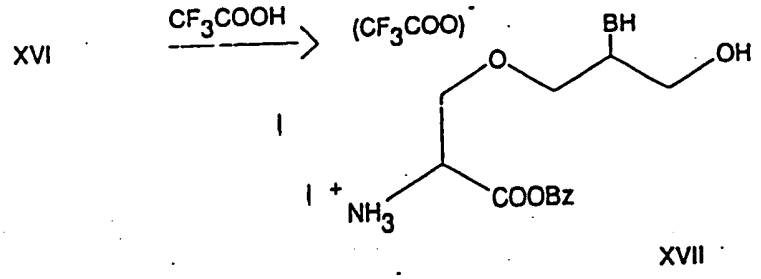
XIII XIV



XIV XV



XV XVI



XVII

This reaction may be carried substantially as follows:

a) BOC-(O-allyl)serine (Compound of formula XIII)

BOC-Serine (X)(16.4 g, 0.08 mol) is dissolved in 100 ml dimethyl formamide (DMF) and cooled with stirring to -5° - 0°. Sodium hydride (7.68 g of 55% material, 0.176 mol)) is slowly added, the temperature rising to about 2°. The reaction mixture is stirred for approx. 1 hr, until no more hydrogen is evolved, then the cooling bath is removed and allyl iodide (8.08 g, 0.088 mol) is added dropwise, the temperature rising to about 43°. After stirring for 1 hr, the reaction mixture is poured into ice water, and extracted twice with ether. The aqueous layer is acidified to pH 3, extracted with ethyl acetate, then saturated with NaCl and again extracted with ethyl acetate. The combined organic extracts are shaken with sodium thiosulphate solution to remove elemental iodine, then dried and evaporated to give the title product as an oil (19.3 g). $[\alpha]_D^{20}$ = +8° (c = 1.1 in CH_2Cl_2)

b) BOC-(O-allyl)serine benzoate (Compound of formula XIV)

The product of step (a) (19 g, 0.077 mol) is dissolved in 400 ml toluene, and warmed to 90°. N,N-dimethylformamide dibenzyl acetal (55ml, 0.19 mol) is added dropwise over 45 min. to give a dark yellow

solution, which is stirred for 1 hr at 90°, then evaporated to give a brown oil which is then purified by chromatography on kieselgel to give the title product as a yellow liquid (20g). $[\alpha]_D^{20}$ = -5.34° (1% in CH_2Cl_2)

c) BOC-(O-2,3-epoxypropyl)serine benzoate (Compound of Formula XV)

The product of step (b) (18g, 53.7 mmol) is dissolved in 200 ml methylene chloride and m-chloroperbenzoic acid (15.4g, 80 mmol) is added. The resulting colourless solution is stirred overnight under argon, to give a white suspension which is filtered, evaporated, and the residue taken up in ether. The ether solution is washed with sodium bicarbonate solution then with brine, then dried and evaporated to give a yellow oil (19 g). After purification by chromatography on Kieselgel, the title product is obtained as a colourless oil (11.4 g), $[\alpha]_D^{20}$ = -5.5° (1% in CH_2Cl_2).

d) BOC-(O-isopropylidene-2,3-hydroxypropyl)serine benzoate (Compound of Formula XVI)

The product of step (c) (10 g, 28 mmol)is dissolved in 100 ml absolute acetone under argon, and cooled to 0°. To the solution is added 360 µl (2.8 mmol) of boron trifluoride etherate, and the resulting yellow solution is stirred for 2 hr at 0°, then evaporated and the residue taken up in t-butyl methyl ether. This solution is washed with $KHCO_3$ solution then with brine, then dried and evaporated to give a yellow oil (12.2 g). Chromatography on Kieselgel gives the pure title product as a pale yellow oil (8.6 g), $[\alpha]_D^{20}$ = -10.7° (1% in CH_2Cl_2).

e) (O-2,3-dihydroxypropyl)serine benzoate trifluoroacetate salt (AA-III in carboxy-protected form)

The product of step (d) (8.0 g, 21 mmol) is dissolved in 50 ml

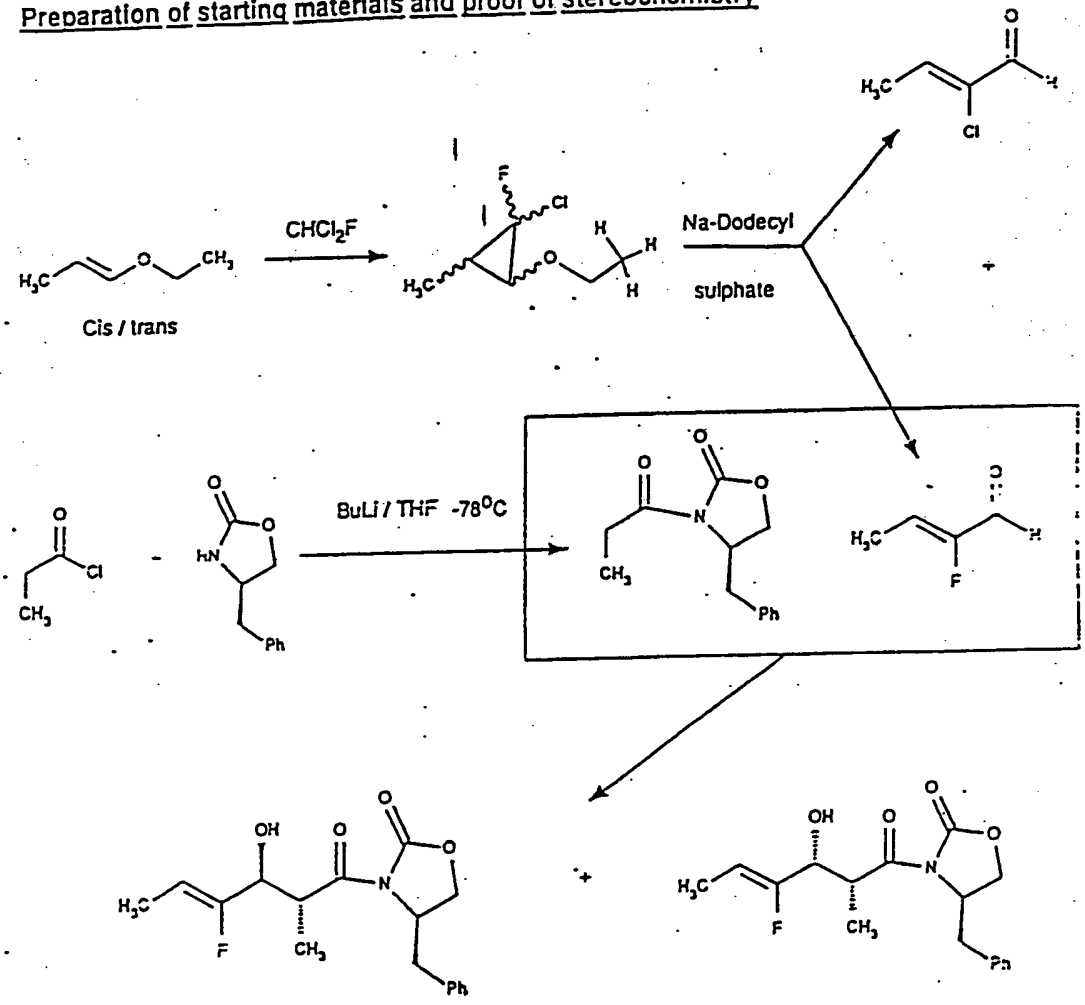
of a 1:4 mixture of trifluoroacetic acid/methylene chloride and stirred 5 hr at room temperature. Evaporation and washing with toluene gives the title product as a yellow oil (7.5 g).

The product may be deprotected, reprotected and coupled by standard procedures.

Example 5-9: Synthesis of halo-olefin isosteres

The following reaction scheme illustrates, by way of example, synthesis routes for chloro- and fluoro-olefin dialanine isosteres in protected form, and subsequent incorporation at various points on an MHC blocker peptide:

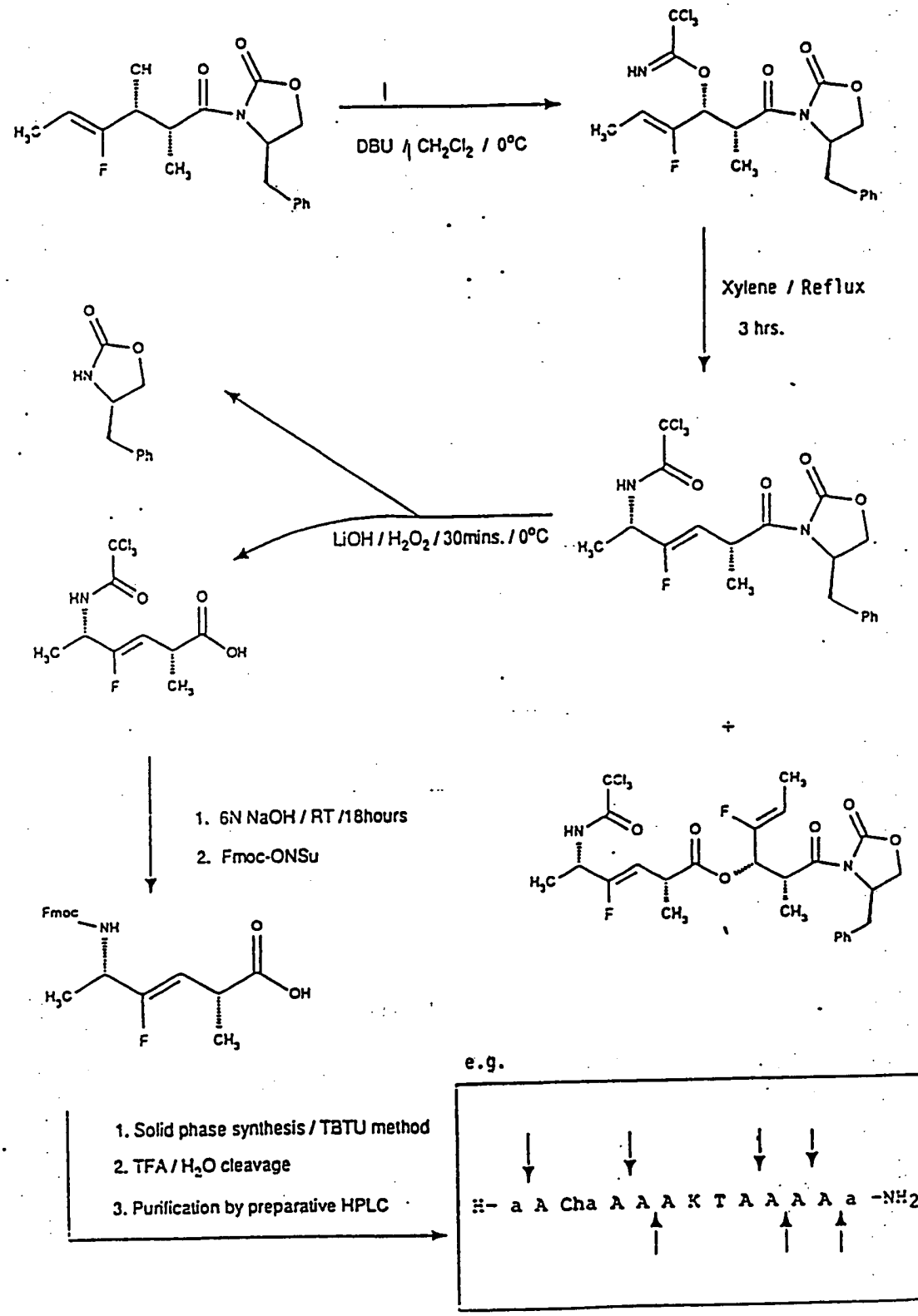
Preparation of starting materials and proof of stereochemistry



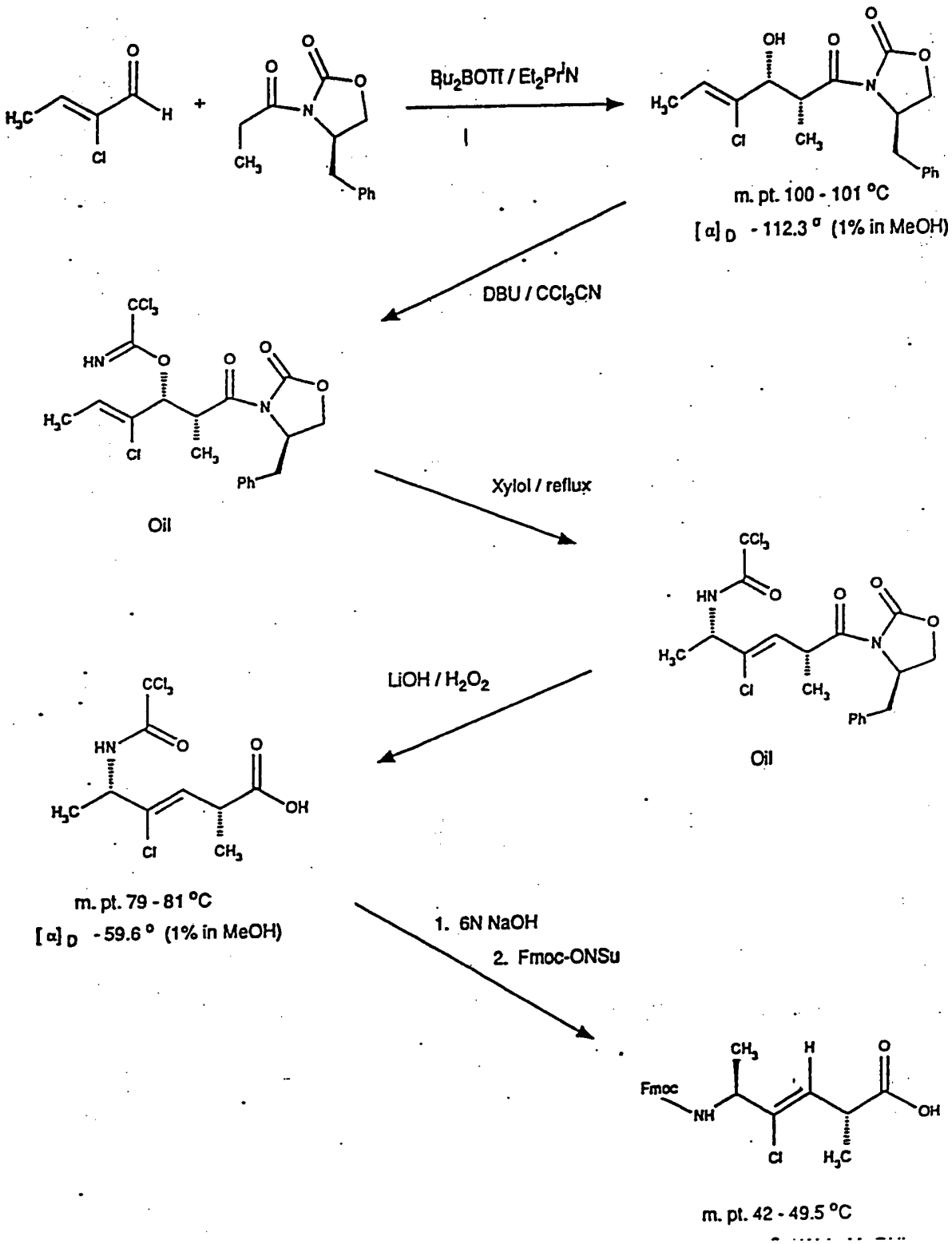
$J_{a,b}$ 10.5 Hz. $J_{Ha,F}$ 22Hz

$J_{a,b}$ < 4Hz. $J_{Ha,F}$ ca 8Hz.

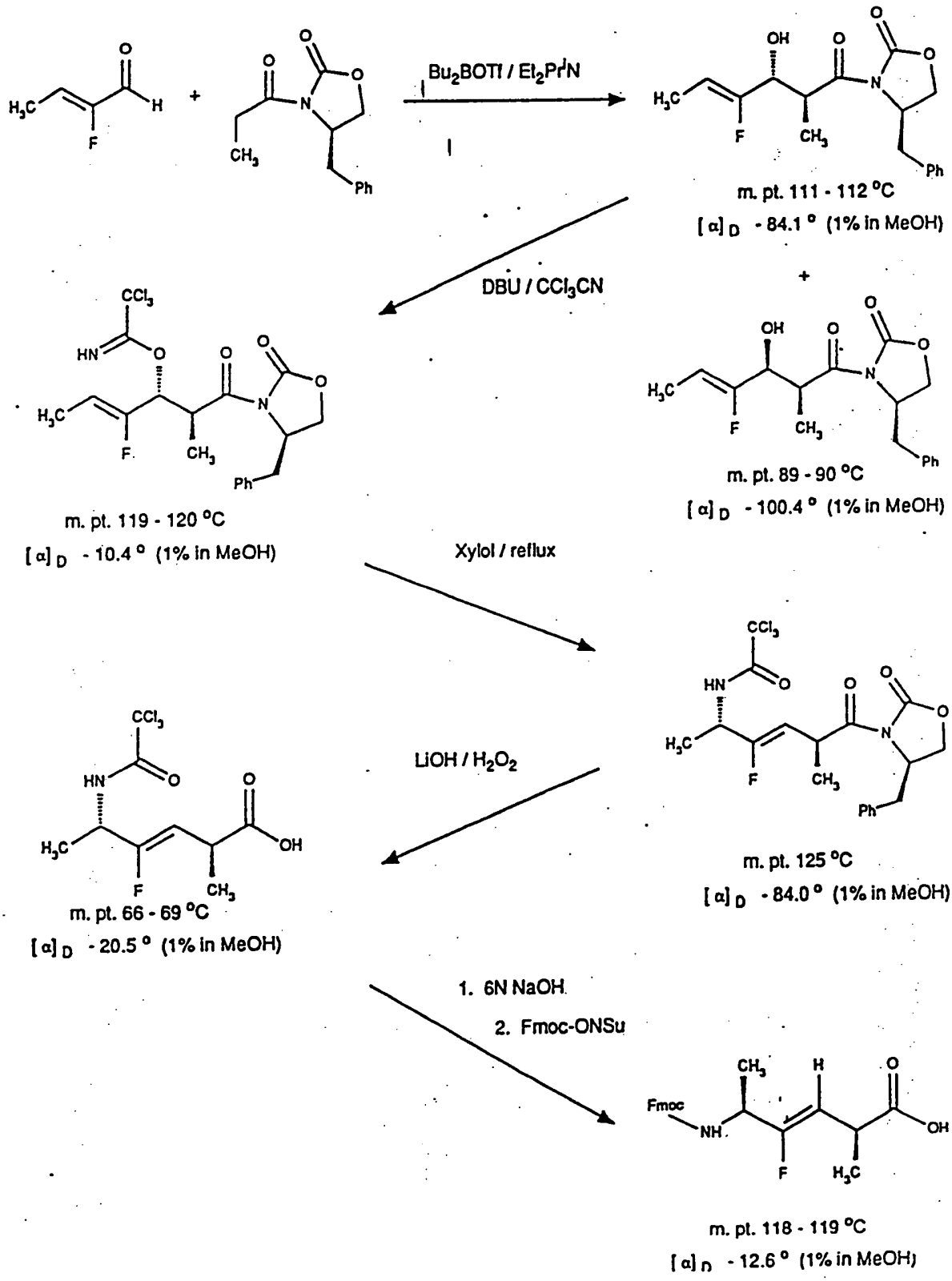
Synthesis of Fmoc-Ala-Ala isoster and its incorporation into MHC blocker peptide



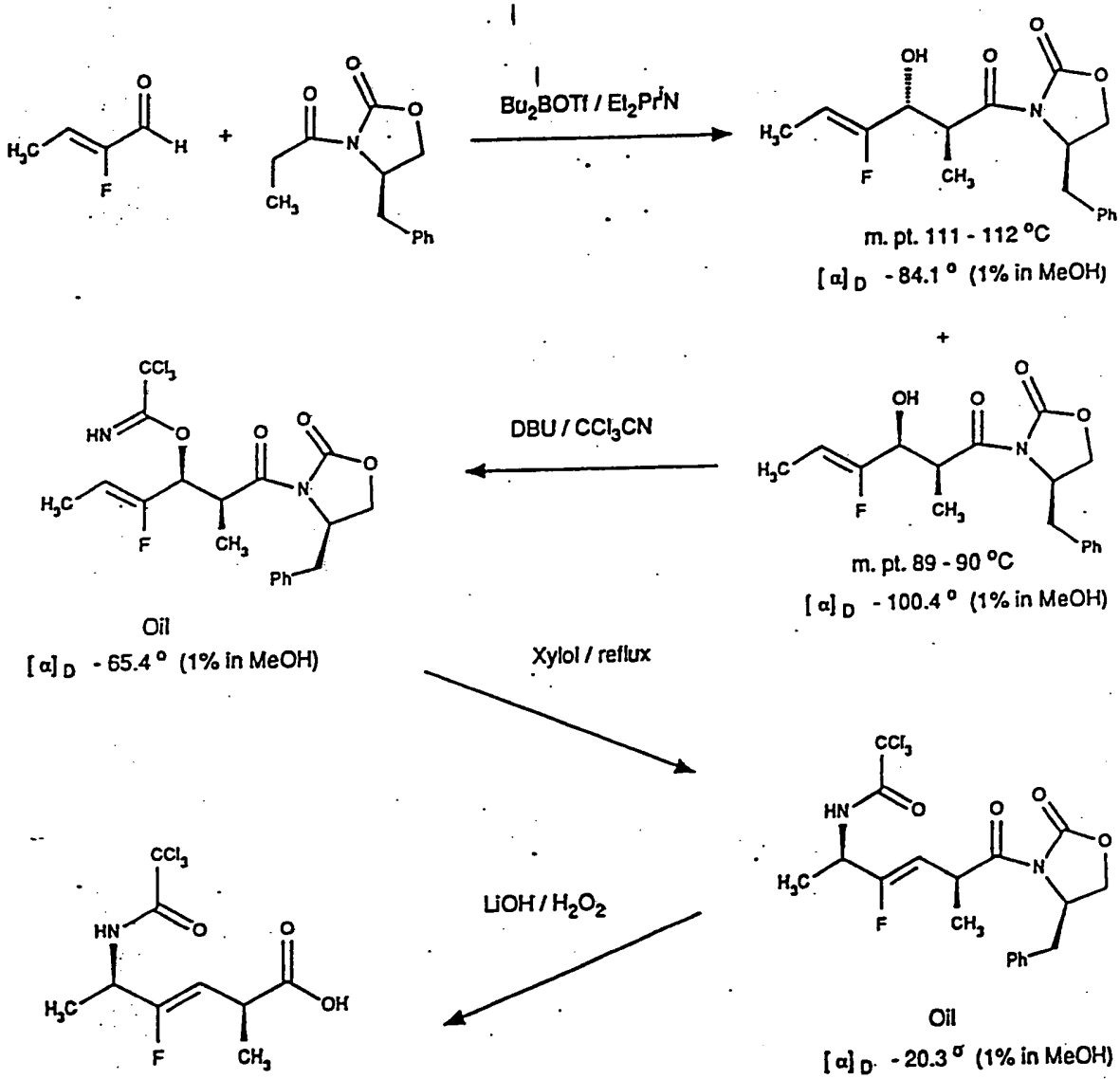
Preparation of Fmoc-Ala{(E)-ClC=CH}Ala isostere



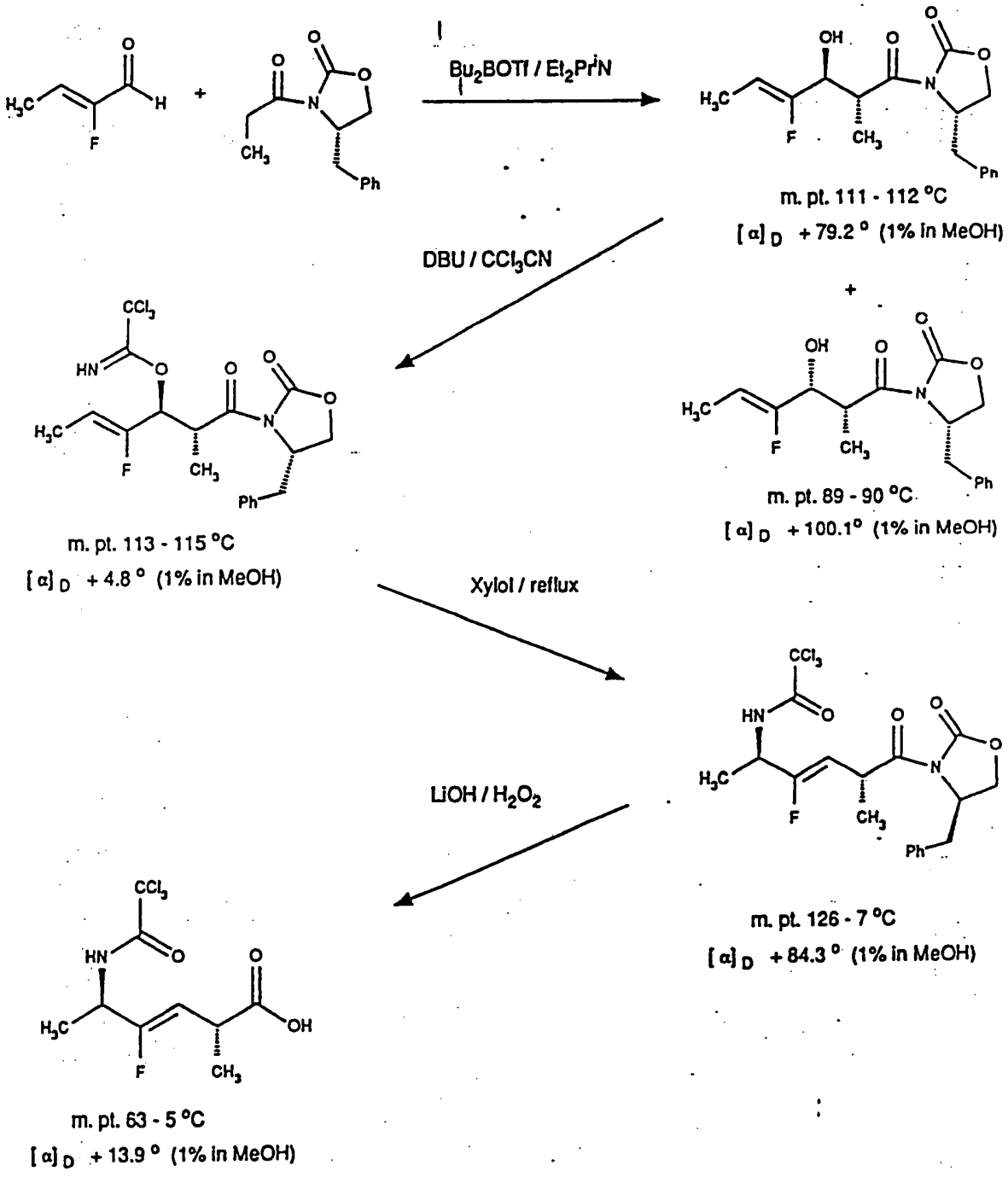
Preparation of Fmoc-Ala{(E)-FC=CH}D-Ala Isostere



Preparation of N-Trichloroacetyl-D-Ala{(E)-FC=CH}D-Ala isostere



Preparation of N-Trichloroacetyl-D-Ala{(E)-FC=CH}Ala isostere



Analogous processes may be used to produce different distereoisomers, different amino acid residue combinations, and different MHC blocker peptides within the scope of the invention. Isosteres of other dipeptides may be synthesized and incorporated into peptides by analogous procedures.

Example 5: Synthesis of Fmoc-Ala{(E)CF=CH}Ala-OH

a) (4R)-3-(1-Oxopropyl)-4-phenylmethyl-2-oxazolidinone.

20% Butyl lithium in hexane is added during 70 minutes to a solution of 51.55g (4R)-4-Phenylmethyl-2-oxazolidinone in 1.5l tetrahydrofuran at -78°C until the colour changed from bright yellow to dark yellow, the mixture stirred for 55 minutes at this temperature, and 26.92g propionyl chloride added over 15 minutes. The mixture is allowed to reach room temperature (280 minutes), decomposed with 500ml saturated $NaHCO_3$ and the solvent evaporated. The residue is partitioned between ethyl acetate and water, the organic phase extracted with water, dried over Na_2SO_4, and evaporated to give a yellow oil which is purified by chromatography over silica gel using 40% diethyl ether/hexane as eluant followed by crystallisation from hexane.

m.pt. 44-45°C, $[\alpha]_D$ -104.3° (1% in ethanol).

b) (4R)-3-[(2R,3R)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone.

150ml dibutylboryl-trifluoromethanesulphonate (1M in methylene chloride) is added over sixty minutes to a solution of 31.9g of the previous product in 500ml methylene chloride at 0°C followed by N-ethyldiisopropylamine over ten minutes. After sixty minutes at this

temperature, the solution is cooled to -78°C and 15.6g (Z)-2-Fluoro-but-2-enal added during ten minutes. The mixture is stirred at -78°C for one hour and then at 0°C for two hours when the boronoic complex is decomposed by treatment with 140ml phosphate buffer (pH 7) followed 550ml methanol and 140ml 30% hydrogen peroxide for thirty minutes. The aqueous phase is extracted with methylene chloride, the organic layer washed with ice cold 1N sodium sulphite followed by water, dried over Na_2SO_4, and evaporated to give a colourless oil. This is chromatographed over silica gel using 25% diethyl ether in hexane to elute the product which is then crystallised from hexane.

m.pt. 98°C $[\alpha]_D$ -120.6° (1% in methanol).

c) (4R)-3-[(2R,5S)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enyl]-4-phenylmethyl-2-oxazolidinone.

1.379g of the product of step b) is dissolved in 6ml methylene chloride, cooled to 0°C and 98mg 1,8-diazabicyclo[5.4.0]undec-7-en(1,5-5) added. This solution is added over ten minutes to a solution of 0.681g trichloroacetonitrile in 2ml methylene chloride at the same temperature. After stirring for 45 minutes at 0°C the solvent is evaporated, the residue dissolved in diethyl ether and chromatographed through silca gel using 10% diethyl ether / hexane to elute the product. This is dissolved in 25ml o-xylene and heated to reflux (ca. 140°C) for approximately three hours until the starting material has disappeared (TLC, absorption at 1660cm^{-1}). The solvent is evaporated, the residue dissolved in toluene and chromatographed over silica gel. The required product is eluted with 2.5% ethyl acetate in toluene and crystallised from hexane. If desired, the structure can additionally be confirmed by X-ray diffraction.

m.pt. 96-97°C. $[\alpha]_D$ -126.5° (1% in methanol).

d) (2R,5S)-(Z)-5-[9-Fluorenylmethoxycarbonyl)-amino]-4-fluoro-2-methyl-hex-3-enoic acid (i.e., Fmoc-Ala-{(E)CF=CH}-Ala-OH)

2.245g of the product of step c) is dissolved in 60ml tetrahydrofuran, cooled to 0°C and hydrolysed with a solution of 0.404g LiOH in 20ml water containing 2.19ml 30% hydrogen peroxide for one hour. The excess peroxide is decomposed with a solution of sodium sulphite and the solvent evaporated. The residue is partitioned between toluene and water, the aqueous phase extracted with toluene followed by diethyl ether and acidified with 2N HCl (pH5), and crystallized from diethyl ether/hexane, the compound having the following properties: m.pt. 85-86°C, $[\alpha]_D$ -84.6° (1% in methanol). 5.05g of this material are dissolved in 100 ml ethanol and hydrolysed overnight under argon with 82ml 6N NaOH. The solvent is evaporated, the residue dissolved in 40ml water, the pH brought to 6 with 35ml 12N HCl, and a solution of 5.57g 9-Fluorenylmethyl-succinimidyl carbonate in 100ml dioxan added. The mixture is stirred overnight at room temperature under argon, acidified with 1N HCl and the organic product extracted with ethyl acetate. After washing to pH 7 the organic phase is dried (Na_2SO_4), evaporated and chromatographed over silica gel. The required product is eluted with 25% ethyl acetate in toluene and crystallised from toluene.

m.pt. 142-144°C $[\alpha]_D$ -48.4° (1% in methanol).

Example 6: Synthesis of Fmoc-Ala{(E)-CCl=CH}Ala-OH

a) (4R)-3-[(2R,3R)-(Z)-4-Chloro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone.

35.4ml. Dibutylboryl-trifluoromethanesulphonate (1M in methylene chloride) is added over sixty minutes to a solution of 7.52g of (4R)-3-(1-Oxopropyl)-4-phenylmethyl-2-oxazolidinone in 150ml methylene chloride at 0°C followed by N-ethyldiisopropylamine (5.83g) over ten

minutes. After sixty minutes at this temperature, the solution is cooled to -78°C and 4.37g (Z)-2-Chloro-but-2-enal added during ten minutes. The mixture is stirred at -78°C for one hour and then at 0°C for two hours when the boronic complex is decomposed by treatment with 35ml phosphate buffer (pH 7), 120ml methanol, and 35ml 30% hydrogen peroxide for thirty minutes. The aqueous phase is extracted with methylene chloride, the organic layer washed with ice cold 1N sodium sulphite followed by water, dried over Na_2SO_4, and evaporated to give a colourless oil. This is chromatographed over silica gel using 10 to 50% diethyl ether in hexane to elute the product, which is then crystallised from diethylether / hexane.

m.pt. 100-101°C $[\alpha]_D$: -112.3° (1% in methanol).

b) (4R)-3-[(2R,5S)-(Z)-4-Chloro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enyl]-4-phenylmethyl-2-oxazolidinone.

6.561g of the product of step a) is dissolved in 30ml methylene chloride, cooled to 0°C and 0.43ml 1,8-Diazabicyclo[5.4.0]undec-7-en(1,5-5) added. This mixture is added over ten minutes to a solution of 3.08g trichloroacetonitrile in 10ml methylene chloride at the same temperature. After stirring for 45 minutes at 0°C, the solvent is evaporated, the residue dissolved in diethyl ether and rapidly filtered through 2x7cm silca gel with methylene chloride / hexane to elute the product. This is dissolved in 600ml o-xylene and heated under reflux for approximately three hours until the starting material has disappeared (TLC, absorption at 1660cm^{-1}). The solvent is evaporated, the residue dissolved in hexane and chromatographed over silica gel. The required product is eluted with 20% diethyl ethyl ether in hexane and used directly in the next reaction.

c) (2R,5S)-(Z)-4-Chloro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enoic acid].

The product of step c) (5.601g) is dissolved in 170ml

tetrahydrofuran, cooled to 0°C, and treated with a solution of 985mg lithium hydroxide in 56ml water for five minutes followed by 5.32ml 30% hydrogen peroxide. After sirring the cloudy solution for 45 minutes at 0°C, the hydrogen peroxide is decomposed with a solution of 5.91g sodium sulphite in 25ml water, the mixture extracted with toluene followed by diethyl ether, the aqueous layers are acidifed with 1N HCl and the product extracted with ethyl acetate. The organic layer is washed with brine, dried over Na_2SO_4, and evaporated to give a residue which could be crystallised from diethyl ether / hexane.

m.pt. 79-81°C $[\alpha]_D$ -59.6° (1% in methanol).

d) (2R,5S)-(Z)-5-[9-Fluorenylmethoxycarbonyl)-amino]-4-Chloro-2-methyl-hex-3-enoic acid (i.e., Fmoc-Ala{(E)-CCl=CH}Ala-OH)

The product of step c) (2.15g) is dissolved in 40ml ethanol, cooled to 0°C and stirred overnight with 33ml 6N NaOH under argon. The solvent is evaporated, the residue dissolved in 40ml water, the pH brought to 5 with 12N HCl and a solution of 2.52g 9-Fluorenylmethyl-succinimidyl carbonate in 50ml dioxan added. The mixture is stirred overnight at 0°C under argon, acidified with 2N HCl, and the organic product extracted with ethyl acetate. After washing to pH 7 the organic phase is dried (Na_2SO_4), evaporated, and chromatographed over silica gel. The required product is eluted with 10% ethyl acetate in toluene and triturated with hexane to give a foam.

m.pt. 42-49.5°C $[\alpha]_D$ -48.4° (1% in methanol).

Example 7: Synthesis of Fmoc-Ala{(E)-CF=CH}ala-OH

a) (4R)-3-[(2S,3R)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone and (4R)-3-[(2S,3S)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone.

A solution of 50ml dibutylboryl-trifluoromethanesulphonate (1M in methylene chloride) is added to a mixture of 11.6g of (4R)-3-(1-

Oxopropyl)-4-phenylmethyl-2-oxazolidinone in 75ml methylene chloride and 10.2ml N-ethyldiisopropylamine at 0°C over thirty minutes. After sixty minutes at this temperature, the orange solution is cooled to -78°C and a mixture of 55ml. diethyl aluminium chloride (1.8M in toluene) in 50ml methylene chloride and 8.81g (Z)-2-Fluoro-but-2-enal at -78°C is added during ten minutes. This is stirred at -78°C for one hour when 150ml phosphate buffer (pH 7) followed by 200ml diethyl ether is added. The aqueous phase is extracted with diethyl ether, the organic layer evaporated and the residue dissolved in 200ml methanol. This is treated with 50ml 30% hydrogen peroxide diluted with 200ml water for one hour, diluted with 200ml water evaporated to dryness at room temperature. The residue is treated with 0.5N HCl and ice cold 1N sodium sulphite, extracted with diethyl ether and washed. The organic layer is dried over Na_2SO_4 and evaporated to give a colourless oil. This is chromatographed over silica gel using 20 - 50% diethyl ether in hexane to elute the products which are then crystallised from diethyl ether - hexane.

(4R)-3-[(2S,3R)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone:

m.pt. 111-112°C $[\alpha]_D$ -84.1° (1% in methanol).

(4R)-3-[(2S,3S)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone:

m.pt. 89-90°C $[\alpha]_D$ -100.4° (1% in methanol).

b) (4R)-3-[(2S,5S)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enyl]-4-phenylmethyl-2-oxazolidinone.

6.3g of the above 2S,3R isomer are dissolved in 30ml methylene chloride, cooled to 0°C and 447mg 1,8-Diazabicyclo[5.4.0]undec-7-en(1,5-5) added. This solution is added over ten minutes to 3.11g trichloroacetonitrile in 15ml methylene chloride at the same temperature. After stirring for two hours at 0°C the solvent is

evaporated, the residue dissolved in diethyl ether and chromatographed through silca gel using 10% diethyl ether / hexane to elute the intermediate imine.

m.pt. 119-120°C $[\alpha]_D$ -10.4° (1% in methanol).

This intermediate is dissolved in 28ml o-xylene and added to 500ml refluxing o-xylene and the mixture refluxed for approximately three hours until the starting material has disappeared (TLC, absorption at 1660cm^{-1}). The solvent is evaporated, and the residue dissolved in ethyl acetate and chromatographed over silica gel (2 x 8cm.). The required product is eluted with ethyl acetate and crystallised from hexane.

m.pt. 125°C. $[\alpha]_D$ -84.0° (1% in methanol).

c) (2S,5S)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enoic acid]

4.2g of the product of step b) are dissolved in 40ml tetrahydrofuran, cooled to 0°C and hydrolysed for thirty minutes with a solution of 0.756g LiOH in 40ml water containing 4.1ml 30% hydrogen peroxide. The excess peroxide is decomposed with sodium sulphite and extracted with toluene. The aqueous phase is acidified with 2N HCl, extracted with ethyl acetate, the organic layer washed with brine, dried over Na_2SO_4, and evaporated. The residue is dissolved in ethyl acetate and filtered through a 2 x 8cm column of silica gel. Evaporation of the solvent yielded the required product which can be crystallised from diethyl ether - hexane.

m.pt. 66-69°C. $[\alpha]_D$ -20.5° (1% in methanol).

d) (2S,5S)-(Z)-5-[9-Fluorenylmethoxycarbonyl)-amino]-4-fluoro-2-methyl-hex-3-enoic acid.

The product of step c) (1.787g) is dissolved in 40ml ethanol and stirred overnight under argon with 29.2ml 6N NaOH. The solvent is

evaporated, the residue dissolved in 40ml water, the pH brought to 9 and a solution of 1.97g 9-Fluorenylmethyl-succinimidyl carbonate in 50ml dioxan added. The mixture is stirred overnight at room temperature under argon, acidified with 2N HCl and the organic product extracted with ethyl acetate. After washing to pH 7, the organic phase is dried (Na_2SO_4), evaporated and the residue dissolved in toluene and applied to a column of silica gel. Elution with 10-50% ethyl acetate in toluene affords the required material which is triturated with hexane.

m.pt. 118-119°C $[\alpha]_D$ -12.6° (1% in methanol).

Example 8: Synthesis of Trichloroacetyl-ala{(E)-CF=CH}ala-OH

a) (4R)-3-[(2S,5R)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enyl]-4-phenylmethyl-2-oxazolidinone.

2g of the 2S,3S isomer of step a) of the previous example are dissolved in 8ml methylene chloride, cooled to 0°C and 142mg 1,8-diazabicyclo[5.4.0]undec-7-en(1,5-5) added. This is added over ten minutes to 988mg trichloroacetonitrile in 4ml methylene chloride at the same temperature. After stirring for one hour at 0°C the solvent is evaporated, the residue dissolved in diethyl ether and chromatographed through silca gel (2 x 7cm) using ethyl acetate to elute the intermediate imine.

oil $[\alpha]_D$ -65.4° (1% in methanol).

This intermediate is dissolved in 15ml o-xylene and added to 300ml refluxing o-xylene and then refluxed for approximately three hours until the starting material has disappeared (TLC, absorption at 1660cm^{-1}). The solvent is evaporated, the residue dissolved in diethyl ether / hexane and chromatographed over silica gel. The required product is eluted with diethyl ether to give an oil.

oil $[\alpha]_D$ -20.3° (1% in methanol).

b) (2S,5R)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enoic acid] (i.e.,

725mg of the product of step a) is dissolved in 7ml tetrahydrofuran, cooled to 0°C and hydrolysed with 131mg LiOH in 7ml water containing 0.71ml 30% hydrogen peroxide for thirty minutes. The excess peroxide is decomposed with sodium sulphite and extracted with toluene. The aqueous phase is acidified with 2N HCl, extracted with diethyl ether and the organic layer washed and dried over Na_2SO_4 and evaporated. The residue is crystallised from diethyl ether - hexane.

m.pt. 85-86°C. $[\alpha]_D$ +84.°1 (1% in methanol).

Example 9: Synthesis of Trichloroacetyl-D-Ala{(E)-CF=CH}-L-Ala-OH

a) (4S)-3-(1-Oxopropyl)-4-phenylmethyl-2-oxazolidinone.

240ml. Butyl lithium in hexan (1.6M) is added during 70 minutes to a solution of 62g (4S)-4-phenylmethyl-2-oxazolidinone in 1.5l tetrahydrofuran at -78°C until the colour changes from bright yellow to dark yellow, the mixture stirred for 30 minutes at this temperature, and 35.6g propionyl chloride added over 10 minutes. The cooling bath is removed, the mixture stirred for one hour at room temperature and then decomposed with 500ml saturated $NaHCO_3$ and the solvent evaporated after stirring for one hour. The residue is partitioned between ethyl acetate and water, the organic phase extracted with water, dried over Na_2SO_4, and evaporated to give a yellow oil which is crystallised from hexane.

m.pt. 44-45°C $[\alpha]_D$ +102.7° (1% in ethanol).

b) (4S)-3-[(2R,3S)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone and (4S)-3-[(2R,3R)-(Z)-4-fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone.

A solution of 27ml dibutylboryl-trifluoromethanesulphonate (1M in

methylene chloride) is added to a mixture of 5.24g of (4S)-3-(1-Oxopropyl)-4-phenylmethyl-2-oxazolidinone in 40ml methylene chloride and 3.34g N-ethyldiisopropylamine at 0°C over fifteen minutes. After 45 minutes at this temperature, the solution is cooled to -78°C and a cold (-78°C) mixture of 20ml. diethyl aluminium chloride (1.8M in toluene) and 3.96g (Z)-2-fluoro-but-2-enal is added during ten minutes. The mixture is stirred at -78°C for four hours when the boronic complex is decomposed by treatment with 20ml 30% hydrogen peroxide and 80ml methanol. During the next 45 minutes, the temperature is allowed to reach 0°C, 200ml brine is added and the aqueous phase extracted with ethyl acetate, the organic layer dried over Na_2SO_4, and evaporated to give a colourless oil. This is chromatographed over silica gel using 5 to 25% diethyl ether in methylene chloride to elute the products which are then crystallised from diethyl ether - hexane.

(4S)-3-[(2R,3S)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone:

m.pt. 111-112°C $[\alpha]_D$ +79.2° (1% in methanol).

(4S)-3-[(2R,3R)-(Z)-4-Fluoro-3-hydroxy-2-methyl-1-oxo-hex-4-enyl]-4-phenylmethyl-2-oxazolidinone:

m.pt. 89-90°C $[\alpha]_D$ +100.1° (1% in methanol).

c) (4S)-3-[(2R,5R)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enyl]-4-phenylmethyl-2-oxazolidinone.

2.915g of the above 2R,3S isomer is dissolved in 15ml methylene chloride, cooled to 0°C and 207mg 1,8-Diazabicyclo[5.4.0]undec-7-en(1,5-5) added. This solution is added over ten minutes to 1.44g trichloroacetonitrile in 5ml methylene chloride at the same temperature. After stirring for two hours at 0°C the solvent is evaporated, the residue dissolved in diethyl ether and chromatographed through silca gel (2 x 7cm) using 10% diethyl ether / hexane to elute the intermediate imine. The product can be crystallised from hexane.

m.pt. 113-115°C $[\alpha]_D$ +4.8° (1% in methanol).

This intermediate is dissolved in 10ml o-xylene and added to 80ml. refluxing o-xylene. The mixture is refluxed for approximately three hours until the starting material has disappeared (TLC, absorption at 1660cm^{-1}). The solvent is evaporated, the residue dissolved in diethyl ether and chromatographed over silica gel. The required product is eluted with 25% diethyl ether in hexane and crystallised from diethyl ether - hexane.

m.pt. 126-127°C. $[\alpha]_D$ +84.3° (1% in methanol).

d) (2R,5R)-(Z)-4-Fluoro-2-methyl-1-oxo-5-(2,2,2-trichloro-1-oxoethylamino)-hex-3-enoic acid] (i.e.,

732mg of the above product are dissolved in 7ml tetrahydrofuran, cooled to 0°C and hydrolysed with a solution of 0.136g LiOH in 7ml water containing 0.71ml 30% hydrogen peroxide for thirty minutes. The excess peroxide is decomposed with a solution of sodium sulphite and extracted with toluene. The aqueous phase is acidified with 2N HCl, extracted with ethyl acetate and the organic layer washed, dried over Na_2SO_4, and evaporated. The residue can be crystallised from diethyl ether - hexane.

m.pt. 63-65°C. $[\alpha]_D$ +13.9° (1% in methanol).

Example 10-12 - Synthesis of cyclic tripeptides

Example 10 - Cyclic (3-1)Lys-Ala-Glu

a) Boc-Lys-Ala-Glu-OMe

Boc-Lys(Z)-Ala-Glu(OBzl)-OMe (0.7 g, 1 mmol), prepared in fully protected form by standard peptide synthesis, is dissolved in 30 ml of methanol and hydrogenated over 10 mg Pd/C for two hours. The catalyst is removed by filtration and the solvent evaporated in vacuo

to obtain ca. 0.4 g of the title compound. MS: (FAB) 461 (MI+H).

b) Cyclic(3-1)-Boc-Lys-Ala-Glu-OMe

Boc-Lys-Ala-Glu-OMe (3.1 g, 6.7 mmol) suspended in 2.5 l of dichloromethane is treated with triethylamine (3.6 ml, 26 mmol) and benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (5.8 g, 14 mmol). The mixture is stirred for 48 hours at 25° C. The solvent is removed in vacuo and the residue triturated twice with 200 ml of diethyl ether. The solid is filtered and chromatographed over silica gel eluting with ethyl acetate/methanol (9:1) to obtain ca. 2.7 g of the title compound.
MS:(FAB) 465 (MI+Na), 365 (MI+Na-Boc).

c) Cyclic(3-1)-Boc-Lys-Ala-Glu

Cyclic(3-1)-Boc-Lys-Ala-Glu-OMe (1.3 g, 2.8 mmol) is dissolved in 45 mL of tetrahydrofuran/water (3:1) and treated with 1N sodium hydroxide (3.2 ml, 3.2 mmol). After two hours at 20°C the pH is adjusted to 6.9 and the solvents are removed in vacuo. The residue is triturated with dichloromethane and dried to obtain ca. 1.3 g of the title compound in sodium salt form. MS :(FAB) 451 (MI+H), 351 (MI-Boc).

d) Cyclic(3-1)-FMOC-Lys-Ala-Glu

Cyclic(3-1)-Boc-Lys-Ala-Glu (1,2 g, 2.8 mmol) is dissolved in 10 ml of dichloromethane/trifluoroacetic acid (1:1). After two hours at 20° C, the solvents are removed under reduced pressure. The residue is dissolved in 20 ml of 10% sodium carbonate and added to a solution of FMOC-ONSU (0.95 mg, 2.8 mmol) in 40 ml of tetrahydrofuran. The reaction mixture is stirred overnight at 20° C. The pH is adjusted to 6.9 with citric acid and the organic solvent is removed under reduced pressure. The precipitate is filtered , washed with water and

triturated twice with ethyl acetate to obtain ca. 1.2 g of the title compound. MS :(FAB) 551 (MI+H).

Example 11 - Cyclic (3-1)Lys-Phe-Glu

a) Boc-Lys-Phe-Glu-OMe

Boc-Lys(Z)-Ala-Glu(OBzl)-OMe (1g), prepared in fully protected form by standard peptide synthesis, is suspended in 40 ml of methanol and hydrogenated by 4 bars over 100 mg Pd/C for two hours at r.t.. The catalyst is removed by filtration and the solvent evaporated in vacuo to obtain ca. 100% of the title compound.

b) Cyclic(3-1)-Boc-Lys-Phe-Glu-OMe

Boc-Lys-Phe-Glu-OMe (470 mg, 0.877 mmol) suspended in 40 ml of dichloromethane and triethylamine (360 mg, 3.56 mmol) is treated with a solution of benzotriazol-1-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (780 mg) in 180 ml dichloromethane in 5 portions until 1 hour by r.t.. The mixture in vacuo and the residue is chromatographed over 70 g silicagel eluting with ethyl acetate/methanol (9:1) to obtain ca. 92% of the title compound, which may then be hydrolized and protected analogously to Example 10 c) and d).

Example 12 - Cyclic (3-1) Orn-Phe-Glu

This is prepared completely analogously to Example 11.

Example 13: Peptides incorporating novel cyclic tripeptides

Example 13: Cyclic(5-3)-Boc-Phe-Ala-Lys-Ala-Glu

Cyclic(3-1)-Boc-Lys-Ala-Glu-OMe (1.2 g, 2.7 mmol) is stirred in

12 ml of dichloromethane/trifluoroacetic acid (1:1) at 20° C for three hours. The solvents are evaporated and the residue is triturated twice with diethyl ether and dried in vacuo. The solid is then redissolved in dimethylformamide (5 ml) containing 1-hydroxy-benzotriazol (0.65 g, 4.9 mmol), N,N-dicyclohexylcarbodiimide (0.87 g, 4.2 mmol) and Boc-Phe -Ala-OH (1.1 g, 3.1 mmol). The reaction mixture is stirred overnight at 20° C and filtered. The solid is triturated 3x with methanol (5 ml), washed with dichloromethane and dried to obtain ca. 1.1 g of cyclic(5-3)-Boc-Phe-Ala-Lys-Ala-Glu-OMe. MS:(FAB) 661 (MI+H), 561 (MI-Boc). The product is soluble in warm dimethyl sulfoxide.

Following the procedure of Example 10(c), the title compound may be prepared by hydrolysis of this product.

a) Cyclic(4-2)-Boc-Pro-Lys-Phe-Glu-OMe

Cyclic (3-1)-Boc-Lys-Phe-Glu-OMe (2.85 mmol) is dissolved in a few volume of ether abs. and stirred with a satured solution (20 ml) of HCl in ether for 2 hours (controled by TLC). The mixture is evaporated in vacuo and the residue is removed and evaporated with 50 ml ether. The final residue is dried over potassium hydroxide in vacuo for 2 hours.

The solid is then redissolved in dimethylformamide (10 ml) containing N-methylmorpholin (288 mg, 2.85 mmol), 1-hydroxy-benzotriazol (500 mg, 3.7 mmol), N,N-dicyclohexylcarbodiimide (763 mg, 3.7 mmol) and Boc-Pro-OH (2.85 mmol). The reaction mixture is stirred overnight at r.t. and filtered (controled by TLC). The filtrat is diluted in 50 ml water and extracted with ethyl acetate. The organic layers were washed with a solution of 10% citric acids and after with a concentrated solution of sodium bicarbonate. The organic layers are dried over magnesium sulfate, filtered and evaporated in vacuo to obtain 76% of

the title compound and the structure comfirmed by and MS, Elemental Analysis, NMR.

The following compounds may be prepared analogously:

Cyclic(4-2)-Boc-Pro-Orn-Phe-Glu-OMe (63%)
Cyclic(4-2)-Boc-(D)Ala-Lys-Phe-Glu-OMe (80%) MS, NMR
Cyclic(4-2)-Boc-(D)Ala-Orn-Phe-Glu-OMe (72%)

b) Cyclic (4-2)-Boc-Pro-Lys-Phe-Glu

Cyclic (4-2)-Boc-Pro-Orn-Glu-OMe (340 mg) is dissolved in 15 ml of tetrahydrofuran and treated with 2N sodium hydroxide (0.5 ml, 2 eq.). After 3 hours at r.t. (control by TLC) the pH is adjusted to 3 (with 2N HCl) and the solvents are removed in vacuo and the structure confirmed by MS, NMR and Elemental Analysis.

The following compounds are prepared analogously:

Cyclic(4-2)-Boc-Pro-Orn-Phe-Glu
Cyclic(4-2)-Boc-(D)Ala-Lys-Phe-Glu
Cyclic(4-2)-Boc-(D)Ala-Orn-Phe-Glu

Example 14: Cyclic(7-5)-Pro-Lys-Phe-Val-Lys-Ala-Glu-Thr -Leu-Lys-Leu-Ala-Thr-NH_2

The product of Example 10(d) is used in a standard solid-phase peptide synthesis as described, e.g., in Example 1, supra.

Example 15: Cyclic(7-5)-Pro-Lys-Phe-Val-Orn-Ala-Glu-Thr -Leu-Lys-Leu-Ala-Thr-NH_2

By analogy with examples 1 and 10, the title compound, in which -Orn- is ornithine (i.e., the 7-5 cyclic tripeptide is a residue of

formula V where A is $-CH_2-CH_2-$) may be produced by solid phase synthesis using Cyclic(3-1)Fmoc-Orn-Ala-Glu.

Example 16: Cyclic(4-2)-Pro-Orn-Phe-Glu-Lys-Gln-Asn-Thr -Leu-Lys-Leu-Ala-Thr

The compound of example 12 is used in a standard solid-phase peptide synthesis as described in example 11 and the structure confirmed by MS.

The following compounds may be prepared analogously:

Cyclic(4-2)-(D)Ala-Lys-Phe-Glu-Ala-Lys-Thr-Ala-(D)Ala-NH_2
Cyclic(4-2)-(D)Ala-Orn-Phe-Glu-Ala-Lys-Thr-Ala-(D)Ala-NH_2

Other amino acid sequences optionally incorporating AA-I, AA-II, AA-III or the halo-olefins of formula IV may be produced by synthesising the desired cyclic tripeptide and building the desired sequence, e.g. as described in example 1.

Example 17: Novel MHC blocker peptides

A variety of novel blocker peptides can be synthesised using the methods described above. Table I lists examples such peptides. Table II shows their MHC binding affinity relative to native peptides with known MHC binding activity. Table III shows the ability of the novel blockers to inhibits MHC restricted antigen dependent T-cell proliferation.

TABLE I

EXAMPLES OF NOVEL MHC BLOCKER PEPTIDES

1.1 tyr-Ala-Ala-Phe-Ala-Ala-Ala-III-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.2 BOC-Ala-I_a-TMSA-Ala-I_a-Ala-I_a-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.3 Ala-I_a-TMSA-Ala-I_a-Ala-I_a-Thr-Leu-Lys-Ala-Ala-ala-NH2

1.4 BOC-Ala-TES-Phe-Ala-TES-Ala-TES-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.5 PEG-Ala-Ala-Phe-Ala-Ala-Ala-Ala-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.6 PEG-Ala-Ala-Phe-Ala-Ala-Ala-TES-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.7 PEG-Ala-Ala-Phe-Ala-TES-Ala-Ala-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.8 PEG-Ala-Ala-Phe-Ala-TES-Ala-Ala-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.9 TES-Phe-Ala-TES-Ala-TES-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.10 tyr-Ala-I-Phe-Ala-I_a-Ala-I_a-Thr-Ala-I_a-Ala-Ala-ala-NH_2

1.11 II-Phe-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.12 II-Phe-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.13 PEG-Ala-Phe-Aib-Ala-Ala-III-Thr-Ala-Ala-Ala-Phe-Thr-OH

1.14 CHP-Ala-I_a-Ala-I_a-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.15 Ala-Ala-TMS-Ala-I_b-Ala-I_b-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.16 CHP-Ala-I_b-Ala-I_b-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.17 CHP-Ala-I_b-Ala-I_b-Thr-Leu-Lys-Ala-Ala-ala-NH_2

2.1 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala- X-X -NH_2

2.2 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- X-X -ala-NH_2

2.3 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.4 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- X-X -Ala-Ala-ala-NH_2

2.5 H-ala-Ala-Cha-Ala- X-X -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.6 H-ala-Ala-Cha- X-x -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.7 H-ala-Ala-Cha- X-X -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.8 H- X-x -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.9 H- X-X -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.10 H- X-X -Cha-Ala- X-X -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.11 H- X-x -Cha-Ala- X-x -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.12 H- X-X -Cha-Ala- X-X -Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.13 H- X-x -Cha-Ala- X-x -Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.14 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala- Z-Z -NH_2

2.15 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- Z-Z -ala-NH_2

2.16 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- Z-Z -Ala-ala-NH_2

2.17 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- Z-z -Ala-ala-NH_2

2.18 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- Z-Z -Ala-Ala-ala-NH_2

2.19 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- Z-z -Ala-Ala-ala-NH_2

2.20 H-ala-Ala-Cha-Ala- Z-Z -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.21 H-ala-Ala-Cha-Ala- Z-z -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.22 H-ala-Ala-Cha- Z-Z -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.23 H-ala-Ala-Cha- Z-z -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.24 H- Z-Z -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.25 H- Z-z -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.26 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- X-X -Thr-OH

2.27 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- X-X -Ala-Thr-OH

2.28 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- X-X -Ala-Ala-Thr-OH

2.29 H-ala-Ala-Cha-Ala-Ala-X-X -Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.30 H- X-X -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.31 H- X-x -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.32 H-ala-Ala-Cha- X-X -Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.33 H-ala-Ala-Cha- X-x -Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

3.1 (7-5) H-Pro-Lys-Phe-Val-Lys-Ala-Glu-Thr-Leu-Lys-Leu-Ala-Thr-NH_2

3.2 (7-5) H-Pro-Lys-Phe-Val-Orn-Ala-Glu-Thr-Leu-Lys-Leu-Ala-Thr-NH_2

3.3 (4-2) H-ala-Lys-Phe-Glu-Ala-Lys-Thr-Ala-ala-NH_2

3.4 (4-2) H-ala-Orn-Phe-Glu-Ala-Lys-Thr-Ala-ala-NH_2

3.5 (4-2) H-Pro-Orn-Phe-Glu-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-OH

wherein

BOC is butyloxycarbonyl;
PEG is heptaethyleneglycol carboxylic acid monomethyl ether,
TES is O-triethyleneglycol serine monomethyl ether,
CHP is cyclohexylpropanoic acid,
Aib is L-aminoisobutyric acid,
TMSA is trimethylsilyl alanine,
Cha is cyclohexylalanine;
I_a is the L-form of the amino acid of formula I where m is zero, n is two and R_a is methyl,
I_b is the L-form of the amino acid of formula I where m is one, n is two and R_a is isopropyl,
II is the L-form of the amino acid of formula II,
III is the L-form of the amino acid of formula III,
NH_2 at the end signifies a carboxyamide modified C-terminus,

and

OH at C-terminus signifies that the carboxy has been reduced to an alcohol.

H signifies an unmodified N-terminus;
X-X is Ala-{(E)CF=CH}-Ala, X-x is Ala-{(E)CF=CH}-ala;
Z-Z is Ala-{(E)CCl=CH}-Ala, Z-z is Ala-{(E)CCl=CH}-ala;

(7-5) indicates a that the side chains of the seventh and fifth residues, counting from the N-terminus, are linked; (4-2) indicates linkage of the fourth and second residues.

TABLE II

BINDING AFFINITY OF NOVEL MHC BLOCKER PEPTIDES

Compound Number	DRI (biotin)	DRI (^{125}I)	DR4v4 (biotin)	DR4v4 (^{125}I)	DR4v14 (biotin)	DR4v14 (^{125}I)
1.2	-	0.1	-	5.0	-	0.7
1.3	-	0.3	-	1.8	-	1.6
1.4	-	0.2	-	3.6	-	0.8
1.5	-	0.2	-	3.9	-	0.9
1.6	-	0.2	-	5.8	-	0.4
1.7	-	0.3	-	5.0	-	0.7
1.8	-	0.3	-	6.2	-	2.0
1.9	-	0.1	-	3.8	-	0.3
1.10	-	0.6	-	10.3	-	-
1.11	4.4	-	17.5	-	-	-
1.12	5.0	-	3.9	-	-	-
1.13	0.7	-	21.0	-	-	-
1.14	0.3	0.0	5.0	4.0	-	1.6
1.15	0.1	0.1	1.7	1.7	-	2.4
1.16	0.0	0.0	2.8	4.6	-	1.6
1.17	0.1	0.1	5.4	9.0	-	7.1
2.1	4.8	31.0	8.8	23.0	-	4.9
2.2	2.8	6.1	2.7	15.0	-	1.1
2.3	1.1	0.2	17.5	8.0	-	0.3
2.4	8.7	3.7	14.0	17.0	-	6.3
2.5	10.4	1.9	17.5	37.0	-	9.3
2.6	3.6	0.4	17.5	13.0	-	0.9
2.7	5.2	5.9	8.0	20.0	-	6.3
2.8	29.0	5.6	25.0	32.0	-	8.7
2.9	12.6	7.8	8.0	34.0	-	6.1

2.14	3.6	22.0	10.3	50.0	-	10.0
2.15	1.4	6.9	5.1	21.0	-	7.9
2.16	0.3	0.3	2.1	6.7	-	0.3
2.17	0.1	0.1	0.6	0.7	-	0.0
2.18	1.6	6.8	11.5	52.0	-	9.2
2.19	0.2	0.4	1.7	5.5	-	0.2
2.20	6.2	8.4	11.5	36.0	-	30.0
2.21	1.4	2.2	7.0	5.9	-	1.3
2.22	2.5	12.0	10.3	52.0	-	14.0
2.23	0.1	0.3	0.4	0.5	-	0.3
2.24	4.9	15.0	10.9	25.0	-	36.0
2.25	5.3	9.9	12.9	38.0	-	15.0
2.26	2.0	-	3.7	-	-	-
2.27	6.7	-	14.0	-	-	-
2.28	1.0	-	3.0	-	-	-
2.29	9.4	-	16.7	-	-	-
2.30	5.3	-	11.2	-	-	-
2.31	18.0	-	20.6	-	-	-
2.32	7.4	-	12.9	-	-	-
2.33	0.1	-	0.1	-	-	-
3.1	1.6	-	2.8	-	-	-
3.2	3.5	-	0.8	-	-	-

Binding affinity for DR1 and DR4 is relative to native peptide A=1, K_D of peptide A is approximately 5.6 nM vs. DR1 and 42 nM vs DR4w4. Peptide A has the sequence:

Pro-Lys-Tyr-Val- Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr.

Binding affinity for DR4w14 is relative to native peptide B = 1. K_D of peptide B is approximately 67 nM. Peptide B has the sequence:

Tyr-Ala-Arg-Phe-Gln-Ser-Gln-Thr-Thr-Leu-Lys-Gln-Lys-Thr.

Results are given where available for both biotin-labelled and radio-labelled (^{125}I) assays, as described above.

TABLE III

CELLULAR ASSAY

Compound Number	DR1	DR4w4	DR4w14
2.1	1.80	0.10	3.07
2.2	1.50	0.60	1.00
2.3	0.40	-	0.03
2.4	0.40	2.70	6.1
2.5	1.30	0.30	1.09
2.6	-	-	-
2.7	0.41	1.50	1.11
2.8	-	3.80	5.5
2.9	0.36	1.00	1.23

This is a competitive assay measuring suppression of proliferation of MHC restricted, antigen specific T-cells, as described above.

Results are given compared to the following reference peptide:

H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-ala-NH_2

which is a peptide invented during the course of the research leading to the present invention, which was found to have extremely high activity in this assay, and thus provides a suitable basis for evaluating the peptides of the invention.

CLAIMS

1. A peptide of formula VI:

$$NX-R_1-P-R_2-T-R_3-CX \quad (VI)$$

wherein

NX is an N-terminal protective group, a residue of a D-amino acid, a residue of an amino acid in protected form, or is not present;

R_1 is a series of from zero to three amino acid residues;

P is a hydrophobic L-amino acid residue or, where NX and R_1 are not present, may be a hydrophobic carboxylic acid residue;

R_2 is a series of three to five L-amino acid residues;

T is a an amino acid residue selected from the group containing L-threonine, L-serine, L-proline, and L-hydroxyproline;

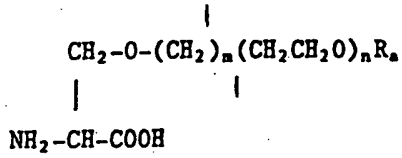
R3 is a series of two to five amino acid residues; and

CX is a residue of an amino acid, an amino acid amide, or an amino alcohol;

which peptide comprises

a) a residue of at least one of the following amino acids:

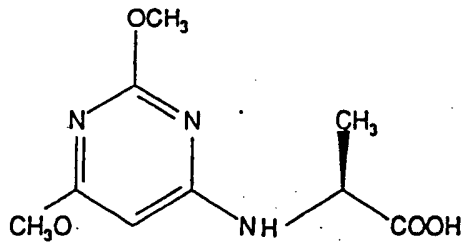
i) the amino acid of formula I (AA-I):



I

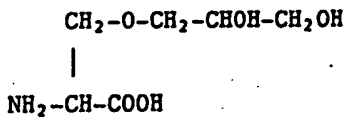
in which m is 0 or 1, n is 1-7 and R_a is C_{1-5} alkyl, provided that when m=0 and n=3, R_a is not methyl;

ii) the amino acid of formula II (AA-II):



II

iii) the amino acid of formula III (AA-III):

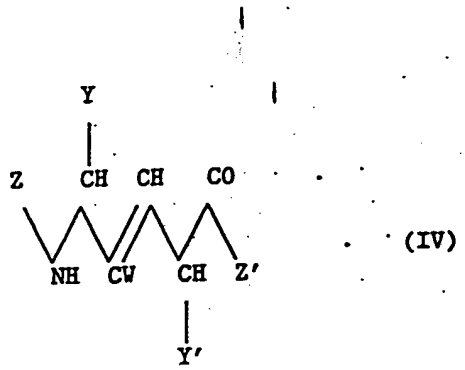


III

iv) O-triethyleneglycol serine monomethylether;

and/or

b) comprises the halo-olefin moiety of formula IV:



(IV)

in which

W is a halogen;

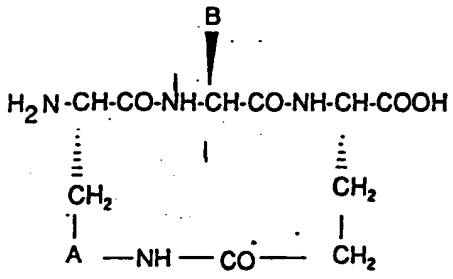
Z and Z' are portions of the peptide of formula VI, so that Z is $NX-R_1-P-R_2-T-R_3$, or a portion thereof, and Z' is $R_1-P-R_2-T-R_3-CX$, or a portion thereof;

Y and Y' are side chains of adjacent residues in the peptide of formula VI; and

the configuration of the olefin bond is trans;

and/or

c) comprises a cyclic tripeptide of formula V:



V

in which

A is a linear C_{2-4} alkylene group, which may be interrupted by an oxygen atom, and B is a side chain of an amino acid.

2. A compound according to claim 1 wherein NX, R_1, P, R_2, T, R_3, and CX of Formula VI are, independently, as follows:

NX is butyloxycarbonyl, fluorenylmethoxycarbonyl, or a residue of D-proline, D-tyrosine, D-alanine, polyethylene glycol carboxylic acid, O-triethyleneglycol serine monomethylether, AA-I, or AA-II, or is not present;

R_1 comprises residues of one or more of the following amino acids: alanine, lysine, O-triethyleneglycol serine monomethylether, AA-I, AA-II, or AA-III, or is not present;

P is a hydrophobic L-amino acid residue selected from the group containing L-phenylalanine and hydrogenated analogues thereof (especially cyclohexylalanine), napthylalanine and hydrogenated analogs thereof, and trimethylsilylalanine (TMSA), or is a residue of cyclohexylpropanoic acid or adamantylacetic acid, or other hydrophobic carboxylic acid, provided that P is a residue of a carboxylic acid, NX

and R_1 are not present;

R_2 comprises residues of four amino acids selected from the group containing glycine, L-threonine, L-alanine, L-lysine, amino-isobutyric acid, O-triethyleneglycol serine monomethylether, AA-I, AA-III, or comprises the cyclic tripeptide residue of Formula V and one other L-amino acid;

T is a residue of L-threonine, L-serine, L-proline, or L-hydroxyproline, preferably L-threonine or L-trans-4-hydroxyproline, most preferably L-threonine;

R3 is a series of two to five residues of the L-forms of one or more of the following amino acids: alanine, leucine, lysine, phenylalanine, O-triethyleneglycol serine monomethylether, AA-I, or AA-III;

CX is a residue of an amino acid, an amino acid amide, or an amino alcohol, preferably L-alaninol, L-threoninol, D-phenylalanineamide or D-alanineamide;

and one or more amide bonds are optionally replaced with a halo-olefin moiety of formula IV.

3. A peptide according to claim 1 selected from:

1.1 tyr-Ala-Ala-Phe-Ala-Ala-Ala-III-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.2 BOC-Ala-I_a-TMSA-Ala-I_a-Ala-I_a-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.3 Ala-I_a-TMSA-Ala-I_a-Ala-I_a-Thr-Leu-Lys-Ala-Ala-ala-NH2

1.4 BOC-Ala-TES-Phe-Ala-TES-Ala-TES-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.5 PEG-Ala-Ala-Phe-Ala-Ala-Ala-Ala-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.6 PEG-Ala-Ala-Phe-Ala-Ala-Ala-TES-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.7 PEG-Ala-Ala-Phe-Ala-TES-Ala-Ala-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.8 PEG-Ala-Ala-Phe-Ala-TES-Ala-Ala-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.9 TES-Phe-Ala-TES-Ala-TES-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.10 tyr-Ala-I-Phe-Ala-I_a-Ala-I_a-Thr-Ala-I_a-Ala-Ala-ala-NH_2

1.11 II-Phe-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.12 II-Phe-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Phe-ala-NH_2

1.13 PEG-Ala-Phe-Aib-Ala-Ala-III-Thr-Ala-Ala-Ala-Phe-Thr-OH

1.14 CHP-Ala-I_a-Ala-I_a-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.15 Ala-Ala-TMS-Ala-I_b-Ala-I_b-Thr-Leu-Lys-Ala-Ala-ala-NH_2

1.16 CHP-Ala-I_b-Ala-I_b-Thr-Ala-I_a-Ala-I_a-ala-NH_2

1.17 CHP-Ala-I_b-Ala-I_b-Thr-Leu-Lys-Ala-Ala-ala-NH_2

2.1 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala- X-X -NH_2

2.2 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- X-X -ala-NH_2

2.3 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.4 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- X-X -Ala-Ala-ala-NH_2

2.5 H-ala-Ala-Cha-Ala- X-X -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.6 H-ala-Ala-Cha- X-x -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.7 H-ala-Ala-Cha- X-X -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.8 H- X-x -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.9 H- X-X -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.10 H- X-X -Cha-Ala- X-X -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.11 H- X-x -Cha-Ala- X-x -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.12 H- X-X -Cha-Ala- X-X -Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.13 H- X-x -Cha-Ala- X-x -Lys-Thr-Ala- X-X -Ala-ala-NH_2

2.14 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala- Z-Z -NH_2

2.15 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- Z-Z -ala-NH_2

2.16 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- Z-Z -Ala-ala-NH_2

2.17 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- Z-z -Ala-ala-NH_2

2.18 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- Z-Z -Ala-Ala-ala-NH_2

2.19 H-ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- Z-z -Ala-Ala-ala-NH_2

2.20 H-ala-Ala-Cha-Ala- Z-Z -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.21 H-ala-Ala-Cha-Ala- Z-z -Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.22 H-ala-Ala-Cha- Z-Z -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.23 H-ala-Ala-Cha- Z-z -Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.24 H- Z-Z -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.25 H- Z-z -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-ala-NH_2

2.26 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala- X-X -Thr-OH

2.27 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr-Ala- X-X -Ala-Thr-OH

2.28 H- ala-Ala-Cha-Ala-Ala-Ala-Lys-Thr- X-X -Ala-Ala-Thr-OH

2.29 H-ala-Ala-Cha-Ala-Ala-X-X -Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.30 H- X-X -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.31 H- X-x -Cha-Ala-Ala-Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.32 H-ala-Ala-Cha- X-X -Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

2.33 H-ala-Ala-Cha- X-x -Ala-Lys-Thr-Ala-Ala-Ala-Ala-Thr-OH

3.1 (7-5) H-Pro-Lys-Phe-Val-Lys-Ala-Glu-Thr-Leu-Lys-Leu-Ala-Thr-NH_2

3.2 (7-5) H-Pro-Lys-Phe-Val-Orn-Ala-Glu-Thr-Leu-Lys-Leu-Ala-Thr-NH_2

3.3 (4-2) H-ala-Lys-Phe-Glu-Ala-Lys-Thr-Ala-ala-NH_2

3.4 (4-2) H-ala-Orn-Phe-Glu-Ala-Lys-Thr-Ala-ala-NH_2

3.5 (4-2) H-Pro-Orn-Phe-Glu-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-OH

wherein

BOC is butyloxycarbonyl,

PEG is heptaethyleneglycol carboxylic acid monomethyl ether,

TES is O-triethyleneglycol serine monomethyl ether,

CHP is cyclohexylpropanoic acid,

Aib is L-aminoisobutyric acid,

TMSA is trimethylsilyl alanine,

Cha is cyclohexylalanine,

I_a is the L-form of the amino acid of formula I where m is zero, n is two and R_a is methyl,

I_b is the L-form of the amino acid of formula I where m is one, n is two and R_a is isopropyl,

II is the L-form of the amino acid of formula II,

III is the L-form of the amino acid of formula III,

NH_2 at the end signifies a carboxyamide modified C-terminus,

OH at C-terminus signifies that the carboxy has been reduced to an alcohol,

H signifies an unmodified N-terminus,
X-X is Ala-[(E)CF=CH]-Ala, X-x is Ala-[(E)CF=CH]-ala,
Z-Z is Ala-[(E)CCl=CH]-Ala, Z-z is Ala-[(E)CCl=CH]-ala,

(7-5) indicates a that the side chains of the seventh and fifth residues, counting from the N-terminus, are linked, and (4-2) indicates linkage of the fourth and second residues.

4. A peptide according to claims 1, 2, or 3 for use as a medicament.

5. A pharmaceutical composition comprising one or more of the peptides of claims 1, 2, or 3 in association with a pharmaceutically acceptable diluent or carrier.

6. A peptide of claims 1, 2, or 3 for use in the manufacture of a medicament for treatment of an autoimmune disease.

7. A peptide of claims 1, 2, or 3 for use in the manufacture of a medicament for the treatment of rheumatoid arthritis.

8. A diagnostic kit comprising one or more of the peptides of claims 1, 2, or 3.

9. The novel amino acids of formula I, formula II, or formula III, as described in claim 1, in protected or unprotected form or in acid or base addition salt form

10. A halo-olefin dipeptide isostere of formula IV as depicted in claim 1 wherein Z is an amino acid residue in protected or unprotected form, a protective group, or hydrogen; Z′ is an amino acid

residue in protected or unprotected form, a protected group, amide or hydroxyl; and Y and Y' are the same or different and are side chains of α-amino acid residues; provided that where Y' is H, Y is other than H or methylbenzyl.

11. A cyclic tripeptide of formula V as depicted in claim 1, in protected or unprotected form, or acid or base addition salt form.

12. A peptide, in protected or unprotected form, incorporating one or more residues according to claims 9, 10 or 11.

13. A process for making a peptide according to claim 12 comprising the steps of

i) synthesizing a compound of formula I, II, II, IV or V in N-terminal protected form;

ii) deprotecting said compound,

iii) reacting the deprotected compound with a peptide in the presence of a coupling reagent, and

iv) isolating the product.

14. All novel products, processes and utilities substantially as disclosed herein.

AMENDED CLAIMS

[received by the International Bureau on 12 January 1993 (12.01.93); original claim 10 amended; remaining claims unchanged (1 page)]

residue in protected or unprotected form, a protected group, amide or hydroxyl; and Y and Y' are the same or different and are side chains of α-amino acid residues; provided that where Y' is H, Y is other than H or benzyl.

11. A cyclic tripeptide of formula V as depicted in claim 1, in protected or unprotected form, or acid or base addition salt form.

12. A peptide, in protected or unprotected form, incorporating one or more residues according to claims 9, 10 or 11.

13. A process for making a peptide according to claim 12 comprising the steps of

i) synthesizing a compound of formula I, II, II, IV or V in N-terminal protected form;

ii) deprotecting said compound,

iii) reacting the deprotected compound with a peptide in the presence of a coupling reagent, and

iv) isolating the product.

14. All novel products, processes and utilities substantially as disclosed herein.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/01995

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07C229/22; C07D239/52; C07C229/30; C07C237/22
C07C237/16; C07K5/02; C07K7/02; C07K7/08

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07C ; C07D ; C07K ; A61K G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[*]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 008 161 (THE BLOOD CENTER OF SOUTHEASTERN WISCONSIN) 26 July 1990 see the whole document ---	1-8
A	EP,A,0 353 732 (CIBA-GEIGY AG) 7 February 1990 see the whole document ---	10,12
A	EP,A,0 230 893 (BRACCO INDUSTRIA CHIMICA) 5 August 1987 see example 19a ---	9
	-/--	

[*] Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 DECEMBER 1992	15. 12. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	p. masturzo

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EMBO JOURNAL vol. 9, no. 6, June 1990, EYNSHAM, OXFORD GB pages 1797 - 1803 T S JARDETZKY ET AL. 'peptide binding to HLA-DR1; a peptide with most residues substituted to alanine retains MHC binding' see the whole document	1-8
A	TETRAHEDRON LETTERS. vol. 32, no. 10, 4 March 1991, OXFORD GB pages 1283 - 1286 M HO ET AL. 'sxynthesis of an ethylene glycol cross-linked amino acid' see the whole document	9
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 28, no. 12, December 1985, WASHINGTON US pages 1766 - 1771 P W SCHILLER ET AL. 'synthesis and activity profiles of novel cyclic opioid peptide monomers and dimers' see the whole document	11,12
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 33, no. 9, September 1990, WASHINGTON US pages 2552 - 2560 A S DUTTA ET AL. 'novel inhibitors of human renin. cyclic peptides based on the tetrapeptide sequence glu-d-phe-lys-d-trp' see the whole document	11,12
A	INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH vol. 37, no. 3, March 1991, COPENHAGEN DK pages 198 - 209 G A HEAVNER ET AL. 'biologically active conformation of thymopentin. studies with conformationally restricted analogs' see the whole document	11,12

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category.°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 103, no. 19, 11 November 1985, Columbus, Ohio, US; abstract no. 160823u, P W SCHILLER ET AL. 'synthesis of side-chain to side-chain cyclized peptide analogs on solid support' page 747 ;column LEFT ; see abstract & INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH vol. 25, no. 2, 1985, COPENHAGEN DK pages 171 - 177 ---	11,12
P,X	JOURNAL OF MEDICINAL CHEMISTRY vol. 34, no. 10, October 1991, WASHINGTON US pages 2125 - 31132 P W SCHILLER ET AL. 'conformational restriction of the phenylalanine residue in a acyclic opioid peptide analogue: effects on receptor selectivity and stereospecificity' see the whole document ---	11,12
P,X	WO,A,9 202 543 (CYTEL CORPORATION & SANDOZ LTD.) 20 February 1992 see the whole document -----	1-8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 92/01995

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see annex

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

The use of undefining terms like "aminoacid residue" or "side chains of adjacent residues" ecc., makes the whole content of the application unclear. Therefore the search has been limited to the subject matter as defined by claim 3; in detail; the peptides of claim 3, the halo-olefin of claim 10 as far as W=Cl or F; Y and Y'= residues of L-Ala or D-Ala; the cyclic tripeptide of claim 11 as far as B=CH3, this being an alarme residue: peptides of claim 12, only isofar as they contain a residue as defined in this note.
Claim 14 was not searched, due to its highly indefinite nature.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9201995 SA 63786

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 02/12/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9008161	26-07-90	AU-A- 4948890	13-08-90
EP-A-0353732	07-02-90	JP-A- 2091051	30-03-90
EP-A-0230893	05-08-87	AU-B- 591225	30-11-89
		AU-A- 6753987	06-08-87
		JP-B- 3064504	07-10-91
		JP-A- 62195388	28-08-87
		US-A- 4916246	10-04-90
		US-A- 4980502	25-12-90
WO-A-9202543	20-02-92	AU-A- 8305491	02-03-92